

PLASMA PROCUREMENT AND CLINICAL EVALUATION  
OF AN INTRAVENOUS IMMUNOGLOBULIN PREPARATION

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This thesis is dedicated to my parents



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## ABBREVIATIONS

The following abbreviations have been used in the text :

Ab	Antibody
Ag	Antigen
AIDS	Acquired immune deficiency syndrome
AIHA	Autoimmune haemolytic anaemia
ALT	Alanine aminotransferase
CFT	Complement fixation test
CMV	Cytomegalovirus
CPT	Conventional(ly) purified tetanus (toxoid)
CVI	Common variable immunodeficiency
ELISA	Enzyme-linked immunosorbent assay
FFP	Fresh frozen plasma
HAV	Hepatitis A virus
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBIG	Hepatitis B immunoglobulin
HES	Hydroxyethyl starch
HIV	Human immunodeficiency virus
HLA	Histocompatibility locus antigen
HNIG	Human normal immunoglobulin
IDDM	Insulin dependent diabetes mellitus
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intramuscular
IPT	Immune purified tetanus
ITP	Idiopathic thrombocytopenic purpura
IU	International unit
IV	Intravenous
MRC	Medical Research Council
NANB	Non-A, non-B
NLH	Nodular lymphoid hyperplasia
PEG	Polyethylene glycol
PEGG	Human placental eluted gammaglobulin
PKA	Prekallikrein activator
RES	Reticulo endothelial system
Rh(D)	Rhesus (D)
SNBTS	Scottish National Blood Transfusion Service
TIG	Tetanus immune globulin
t <sub>1/2</sub>	Half-life
VZIG	Varicella zoster immunoglobulin
WHO	World Health Organisation
XL	X-linked
ZIG	Zoster immune globulin

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# DECLARATION

I declare that this thesis has been composed by myself and that the work presented here is my own, except where stated in the text.

1 SUMMARY

The studies carried out in this thesis examined various aspects of passive immunisation against infection, from immune plasma procurement to evaluation of an immunoglobulin preparation. Strategies for identifying blood donors suitable for donating plasma for the manufacture of three specific hyperimmune globulins - hepatitis B (HBV), cytomegalovirus (CMV) and tetanus antitoxin immunoglobulins - and the use of an intravenous immunoglobulin (IV IgG) preparation, Scottish National Blood Transfusion Service (SNBTS) IV IgG, in patients with primary hypogammaglobulinaemia were examined.

The screening of 29,265 serum samples identified 19 donors with high HB surface antibody levels. Of the 10 donors who were plasmapheresed, only two were still donating plasma regularly one year later. Similarly, of 845 donors screened, 85 (10%) had suitably high serum CMV antibody levels ; 48 donors were plasmapheresed, but only 12 remained suitable donors one year later. These studies confirm that random screening is an inefficient means of identifying hyperimmune plasma donors. The frequency of unselected blood donors with high tetanus antibody levels is likewise low (1.5% to 2.8%), so that other methods of identifying hyperimmune donors need to be examined. Following a comparative trial of two tetanus toxoids, hyperimmune donors were plasmapheresed. The results

show that individuals can be hyperimmunised without an excess of adverse reactions and that donors most suitable for hyperimmunisation were young males, preferably under 25 years of age, with a preimmunisation tetanus antibody level of around 8 IU/ml. Hyperimmunisation of suitable donors with vaccines should, therefore, be considered prior to plasmapheresis. However, while this may be feasible for hepatitis B plasma procurement, the concern over the safety of currently available CMV vaccines means that repeated random screening remains the only means of identifying CMV plasma donors.

Studies on the use of SNBTS IV IgG (manufactured from Cohn fraction II with subsequent ultrafiltration and mild pepsin proteolysis at pH4) in patients with primary hypogammaglobulinaemia demonstrated that the product was well tolerated even when administered in high doses. Ten of the 25 patients studied experienced adverse reactions during 34 of the 738 infusions (6.4% of all infusions) and all but four were mild; 21 (62%) of adverse reactions were encountered within the first five infusions in each of the patients. In 13 of 17 patients studied, treatment with individualised dosage of SNBTS IV IgG resulted in a trough serum IgG levels of greater than 4 g/l. Most patients benefitted from an increased serum IgG level although the frequency of diarrhoea was



unaffected and one patient with severe bronchiectasis continued to suffer from respiratory infections. There was no evidence of viral hepatitis transmission in 16 patients studied who received SNBTS IV IgG over six to 25 months, as measured by serial serum alanine aminotransferase (ALT) levels. SNBTS IV IgG is therefore a safe and effective therapy for patients with primary hypogammaglobulinaemia.

2    AIMS OF THE THESIS

The treatment of infectious diseases depends mainly on the use of antimicrobial agents. Where these agents are either unavailable or ineffective, specific IgGs may be particularly helpful, either as therapy or prophylaxis. IgG replacement therapy has been used in patients with hypogammaglobulinaemia and has improved their long-term outcome.

This thesis examines the procurement of hyperimmune plasma for the production of IgG used in the prophylaxis and treatment of certain infections. It also deals with the clinical evaluation of a new IgG preparation designed for safe IV use.

Source plasma for the manufacture of specific hyperimmune globulins is obtained from hyperimmune or convalescent donors. The recruitment of such donors has until recently been limited to random screening of large donor populations to identify those donors with adequate antibody level. Alternatively, donors can be hyperimmunised with specific vaccines to render them suitable for donating hyperimmune plasma. However, this is only possible if a safe vaccine is available. The feasibility of recruiting hyperimmune plasma donors for the manufacture of tetanus, hepatitis B and CMV hyperimmune globulins either by random screening or by specific hyperimmunisation has been examined in this thesis.

There is no doubt that patients with primary

hypogammaglobulinaemia benefit from IgG therapy, and until recently this has been mainly in the form of intramuscular (IM) IgG injections and of fresh frozen plasma (FFP) infusions. The IV administration of IgG has been associated with a high incidence of adverse reactions, particularly in patients with primary hypogammaglobulinaemia. However, many clinicians believe that this route of administration offers more advantages over conventional therapy with IM gammaglobulin, due to the higher levels of IgG administered. As a result, new manufacturing processes have been developed over the last few decades for the production of safe IV IgG preparations. One such process is the mild pepsin proteolysis at pH4 method that has been adopted by the Protein Fractionation Centre of the SNBTS.

This product was evaluated in terms of its safety, tolerance and efficacy in patients with primary hypogammaglobulinaemia. Safety and tolerance studies were based on the frequency of adverse reactions and on the incidence of hepatitis events defined as abnormally elevated ALT levels in the recipients. Attempts were made at increasing the serum IgG into the normal range in patients with primary hypogammaglobulinaemia by individualising the dose of IV IgG administered and the clinical response to the higher serum IgG level was assessed in some patients.

### 3 INTRODUCTION

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### 3.1 HISTORY OF HUMAN SEROTHERAPY

In 1890, nearly a century after Edward Jenner introduced vaccination, Emil Adolf von Behring and Shibasaburo Kitasato developed an antitoxin against diphtheria. In the following year, a little girl suffering from diphtheria was successfully treated in a Berlin clinic with diphtheria antitoxin and the era of passive immunisation was born. Specific antisera raised in animals were subsequently used in the treatment of many other serious infections, including rabies, tetanus and anthrax.

As the use of animal antisera spread, hypersensitivity reactions to sera were increasingly described, both in animals (Simon Flexner) and in man. In 1902, Charles Robert Richet, Professor of Physiology in Paris, described anaphylaxis after he observed violent symptoms of poisoning, followed by death within 12 to 24 hours in subjects receiving repeated injections of foreign animal protein substances. Other allergic reactions were later described by Nicolas Maurice Arthus and Charles Peter von Pirquet. With the increasing awareness of reactions to animal antisera, research was directed towards the concentration and purification of serum. It was felt that inactive protein devoid of protective value caused sensitisation and that removal of the inactive protein would yield a safer product (reviewed by Parish, 1965 ; and Janeway, 1970).



In 1910, Berry Gibson and Edwin Banzaf introduced salt fractionation to concentrate the antitoxic serum. Although reduced, the incidence of reactions to the purified antiserum remained significant and an alternative source of serum was sought. Interest was focussed on rabbit antiserum because rabbit antibody globulin was felt to be less likely to cause adverse reactions, by virtue of its physical properties. However, Frank Lappin Horsfall found that the incidence of adverse reactions associated with the use of rabbit gammaglobulin was still significant.

Material derived from humans was eventually used in an attempt to further reduce the incidence of reactions. In the early 1930s, Charles F McKhann and co-workers produced a globulin extract using ground human placenta as a source of human antibody. By 1935 these fractions were shown to be effective in the prevention or modification of measles (McKhann et al, 1933 ; 1935). They were therefore referred to as 'human immune globulin'.

In the late 1930s, studies by Heidelberger and Petersen with the ultracentrifuge, and by Tiselius and Kabat with electrophoresis in liquid media, showed that antibodies belonged to that globulin fraction of serum proteins of slow mobility (at that time designated gammaglobulins).

By the early 1940s, Edwin Joseph Cohn and colleagues

in Harvard developed the method of large-scale cold ethanol fractionation. This method, which is still used today, has three main advantages : firstly, it uses a cheap, volatile compound (alcohol) as a precipitant and therefore allows the work to be done below the freezing point of water, thus minimising the risk of microbial multiplication or denaturation ; secondly, the alcohol can be removed rapidly from the relatively small volume of precipitated protein by lyophilisation ; and thirdly, it is possible to manipulate five different variables, allowing maximal opportunity for separating one protein from another on the basis of solubility difference.

Cohn was also the first to suggest using convalescent hyperimmune plasma as a source of material for specific antimicrobial antibodies. The first two practical attempts were made during World War II. Convalescent scarlatina plasma was collected by Thalhimer for use in man, but became obsolete with the introduction of penicillin. Stokes collected convalescent mumps plasma, which was shown by McGuinness and Peters to be more effective than standard gammaglobulin in the prevention of mumps orchitis in mumps epidemics among soldiers. During the Korean war, Cohn saw the possibility of repeated plasmapheresis of specially selected donors, and Stokes and Smolens carried out plasmapheresis using donors hyperimmunised with mumps

and pertussis vaccine. Since then, plasmapheresis by various techniques has been widely used for the manufacture of human hyperimmune gammaglobulin preparations.

Early in the clinical use of gammaglobulin, it became apparent that the IV administration of Cohn II gammaglobulin fraction to sick children was associated with severe adverse reactions. Stokes gave a large dose of gammaglobulin intravenously to a child with measles encephalitis, who had subsequently developed marked hyperpyrexia and convulsed. Similarly, Janeway observed vasomotor collapse, restlessness, chills and hyperpyrexia in an adolescent with severe measles following the IV administration of gammaglobulin. Thus, the IV administration of gammaglobulins was abandoned.

The recognition of the syndrome of primary hypogammaglobulinaemia by Bruton in 1952, and of the need of such patients for large prophylactic IgG replacement, led to further work on the safety of gammaglobulin administration. The IM gammaglobulin preparation was viscous and caused pain following injection. Large volumes of the immune globulin were needed to maintain adequate blood levels, and during periods of infection when IgG losses were higher more frequent injections were required. Complications were not uncommon : the large injections caused muscle

damage, nerve injury and sterile, as well as pyogenic, abscesses. As a result, patient compliance was poor and their clinical condition did not improve. Other routes of administration were explored, including the subcutaneous infusion of gammaglobulin via a constant infusion pump (Berger et al, 1980). However, the need to find a suitable alternative was still present.

Progress in this field depended on understanding the cause of adverse reactions to the gammaglobulins. In 1962, Barandun and co-workers thought that reactions to gammaglobulin were linked to the anticomplementary activity of the gammaglobulin preparations. As a result, various workers have attempted to modify the concentrated gammaglobulin so that it would not aggregate and activate complement, and at the same time leave both the Fc and Fab parts of the molecule biologically intact. However, such manoeuvres may alter the function and half-life of the whole gammaglobulin molecule, rendering them unacceptable for clinical use (Janeway et al, 1968).

Today, after scientists have grappled with the problem for at least 30 years, numerous IgG preparations are now available for IV use. However, although the problems of adverse reactions have been minimised and gammaglobulins can be safely administered intravenously in large amounts, other problems have emerged. The reported outbreaks of non-A, non-B (NANB) hepatitis in

patients with primary hypogammaglobulinaemia receiving certain IV IgG preparations (Lever et al, 1984 ; Ochs et al, 1985) have raised new questions regarding the safety of IV IgG over the transmission of viral infections.

### 3.2 FUTURE TRENDS

Since Kohler and Milstein (1975) published the first examples of a general method of making monoclonal antibodies in large quantities, new clinical indications for IgGs have emerged : cancer and leukaemia immunotherapy, passive antibody to treat infections and immune regulation. The potential of monoclonal antibodies is enormous, and scientists are only now seeing the beginning of their impact on the study and treatment of disease ; serotherapy with monoclonal antibodies has taken on new dimensions.

However, the administration of murine monoclonal antibodies from murine hybridomas appears to be of limited value at present, since the species of origin of the monoclonal antibody severely restricts their biological usefulness in vivo. The prospect of obtaining human monoclonals will expand the potential clinical application of these reagents, not only for the treatment of infections but also for the possible modulation of the human immune response. Furthermore, it may be possible to link monoclonal antibodies with chemotherapeutic agents for the treatment of cancer (see Section 3.4.9).

### 3.3 ACCEPTED CLINICAL USE OF IMMUNOGLOBULIN

There are well-established and proven uses of IgG preparations, mainly in the prophylaxis of viral diseases and in the neutralisation of bacterial toxins. These have been reviewed by McClelland and Yap (1984). Basically, there are two main types of IgGs for clinical use ; normal, and specific IgGs (Table 3.3.1).

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#### HUMAN IMMUNOGLOBULIN PREPARATIONS

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##### NORMAL IMMUNOGLOBULIN

- (a) intramuscular preparation
- (b) intravenous preparation

##### SPECIFIC IMMUNOGLOBULINS

- (a) Hepatitis B
  - (b) Rubella
  - (c) Varicella-Zoster
  - (d) Rabies
  - (e) Tetanus
  - (f) Rh(d)
  - (g) Cytomegalovirus
- 

Table 3.3.1

#### 3.3.1 Hepatitis A (HAV)

The prophylactic value of human gammaglobulin against viral hepatitis was first demonstrated in 1944 by Stokes and Neefe, who prevented the spread of an epidemic of HAV in a children's summer camp (Stokes and Neefe, 1945). This was later confirmed by two other studies carried out in adults in the United States armed

forces (Gellis et al, 1945), and in institutionalised children (Havens and Paul, 1945). Human normal immunoglobulin (HNIg) is protective against HAV if administered any time during the incubation period, and up to six days before the onset of disease.

Protection is thought to last for six to eight weeks, although Stokes et al (1951) found that a single dose of HNIg (0.02 ml/kg bodyweight) provided some protection for up to nine months for persons living in an institution where HAV was endemic.

The degree of protection varies from 80% to 95%, depending on how soon HNIg is administered after exposure (Grady, 1975). HNIg suppresses the clinical manifestation of the disease, and the incidence of anicteric hepatitis may be 12 times that of icteric hepatitis (Krugman et al, 1960).

Post-exposure prophylaxis is recommended for contacts in private households (both temporary and permanent members), and for contacts in large institutions such as prisons or institutions for the mentally handicapped. However, transmission of the disease is uncommon in casual contacts of a single case in schools, offices or hospitals and HNIg is therefore not recommended for routine use in this setting, unless in the case of an obvious epidemic.

Pre-exposure prophylaxis : Handlers of non-human primates are at risk of HAV, and individuals working



directly with newly-imported primates should receive HNIg at four- to six-monthly intervals. In addition, HNIg has been shown to be of benefit and is routinely recommended to travellers to tropical areas and developing countries where sanitation is substandard. The dose is 750 mg, or 8 mg/kg bodyweight of HNIg at four- to six-monthly intervals.

### 3.3.2 Hepatitis B (HBV)

The first trial using hepatitis B immunoglobulin (HBIg) was carried out by Krugman and his colleagues in 1971, with HBIg prepared from plasma containing high titre anti-HB Ab obtained from a single donor with haemophilia. Of 10 children who received the HBIg, six were completely protected and one of the remaining four developed an attenuated illness (60-70% protection). Later studies by the same group of workers confirmed that HBIg could significantly reduce the incidence, severity and carrier rate of hepatitis B surface antigen (HBsAg) after parenteral exposure to HBV (Krugman et al, 1971 ; 1973).

Other studies examining the use of HBIg for prophylaxis against HBV infection have been conflicting for a variety of reasons. There were differences in study designs, in populations studied, in the methods, intensity and duration of follow-up evaluation and in the assays and criteria chosen to diagnose hepatitis.

Finally, there were large variations in the amount of anti-HBV antibody contained in the HBIg preparations used. Nonetheless, it can be inferred that (1) HBIg given promptly after exposure reduces the incidence of clinical hepatitis ; (2) HBIg is effective in preventing mother-to-infant transmission of HBV ; and (3) HBIg should be administered promptly, probably within three days of exposure, to be effective.

Post-exposure prophylaxis : Indications for HBIg post-exposure prophylaxis depend on the risk of contracting HBV infection, as assessed by the nature of the exposure and from the HBV status of the infecting source. Other indications include babies born to HBsAg-positive mothers, regular sexual contacts of carriers and cases of HBV and accidental transfusion of HBsAg-positive blood or blood products (Morbidity and Mortality Weekly Report, 1981). HBIg has no place in the treatment of ongoing infection, even if administered in large doses (Acute Hepatitis Failure Group, 1977). The recommended dose is 8-11 mg/kg bodyweight.

Whenever immediate protection is required, as for example for infants born to HBsAg-positive mothers or following transfer of an individual into a high-risk setting or after accidental inoculation, active immunisation with the vaccine should be combined with simultaneous administration of HBIg at a different site (Zuckerman, 1986). The presence of passive antibodies

does not impair the development of an active antibody response to the vaccine (Szmuness et al, 1981a ; Deinhardt et al, 1981).

Wong et al (1984) found that vaccine plus HBIG appeared more effective than vaccine alone in preventing the perinatal transmission of HB virus, but the results were not statistically significant. Giving HBIG alone in these circumstances often merely delayed the appearance of chronic antigenaemia (Reesink et al, 1979).

#### 3.3.3 Non-A, non-B (NANB) Hepatitis

Kuhns et al (1976) found HNIg to be of no benefit in the prevention of NANB hepatitis. However, later studies by Seeff et al (1977) and Knodell et al (1976) showed that HNIg reduced the incidence of NANB post-transfusion hepatitis. It is therefore suggested that HNIg should probably be used for post-exposure prophylaxis following needle-stick injuries involving clinical cases of NANB hepatitis.

#### 3.3.4 Measles

Measles may be a severe illness in young children who are either immunocompromised or severely malnourished. Early studies have established the efficacy of convalescent serum or placental extracts in the prevention or modification of measles (Zingher, 1924 ; Park and Freeman, 1926; McKhann et al, 1935). The

degree of protection was found to be dose-dependent; large doses of HNIg given immediately after exposure could prevent measles and small doses only modified the illness (Stokes et al, 1944 ; Ordman et al, 1944). There was also a reduced incidence of encephalitis in measles modified by HNIg (Greenberg et al 1955). Children who are immunocompromised as a result of malignancy, or of immunosuppressive therapy, should receive a large dose of HNIg (0.5 ml/kg bodyweight, maximum of 15 ml) as soon as possible after exposure.

Krugman et al (1962) have shown that HNIg could reduce the incidence of adverse reactions to the Edmonston strain of attenuated measles vaccine. The incidence of high fever and rash was reduced from 40% to 14% and 10% to 2 % respectively. HNIg is also used to prevent adverse reactions to measles vaccine in children with a past history of convulsions or with a family history of idiopathic epilepsy, but the dose used is very much smaller than when used in passive immunisation of immunocompromised children (less than 200 times smaller).

### 3.3.5 Rubella

Congenital rubella is a consequence of maternal viraemia, and immunoprophylaxis has a very limited role in its prevention. Schiff (1969) found that 20 ml of high-titred rubella IgG could prevent

viraemia. Pollack (1969) and the Public Health Laboratory Service Working Party (1970) have shown that HNIg cannot be relied upon to prevent viraemia, particularly in clinical practice where it is often very difficult to establish the timing of exposure to the virus. Other studies using rubella IgG have failed to demonstrate significant protective effect in congenital rubella.

Attempted prophylaxis is therefore restricted to pregnant women who are exposed to rubella and in whom therapeutic abortion is excluded for any reason ; the largest acceptable dose (10-20 ml) should then be given.

### 3.3.6 Varicella zoster

There is no evidence that HNIg can prevent chickenpox, but many studies have shown that HNIg given in large doses can modify the severity of the illness (Trimble, 1957 ; Ross, 1962 ; Iriarte et al, 1965).

Brunell and co-workers have shown that high-titred IgG prepared from patients convalescing from chickenpox or herpes zoster can protect children against chickenpox, but larger doses are required to prevent or modify the disease in the immunosuppressed (Brunell et al, 1969 ; 1972). Furthermore, zoster immune globulin (ZIG) could allow subclinical infection associated with the acquisition of passive-active immunity (Gershon, 1974). The plasma used to produce this hyperimmune

globulin has been in short supply and a suitable alternative is to collect plasma from donors found to have high antibody titres to varicella zoster virus during antibody screening programmes. The IgG produced from the latter source of plasma, called varicella zoster immunoglobulin (VZIg), has been shown to be equally, if not more, effective in protecting immunosuppressed children from severe chickenpox (Zaia et al, 1983).

VZIg is used for the passive immunisation of susceptible immunocompromised patients and premature babies after significant exposure to varicella virus. It should be given as soon as possible after exposure but may be effective when given as late as 96 hours after exposure.

### 3.3.7 Rabies

Rabies is probably the ideal infection for post-exposure prophylaxis by passive immunisation since the exact time, nature and source of exposure are usually identifiable and the long incubation period enhances the effectiveness of passively administered antibody.

Habel (1945) demonstrated that passively administered antibody in experimental rabies neutralised the virus in the tissues, and slowed the spread of the virus within the nervous system, and increased protection by allowing active immunity by vaccine to

become better established. Rabies IgG should only be used with active rabies vaccine to provide protective antibody during the period before the active immune response develops (Baltazard, 1955). However, when given with the vaccine, the antibody response to the vaccine is reduced (Loofbourow et al, 1971). Smaller doses are therefore used to achieve passive-active immunity; doses of 20 IU/kg bodyweight do not inhibit the immune response to a course of three or more doses of human diploid cell vaccine and should not be exceeded (Mertz et al, 1982).

Until 1971, equine antirabies serum was the only preparation available for use. Human rabies IgG has now been produced in some centres. It is very safe and has a circulation half-life twice that of equine antibody. It should be given IM and by local infiltration round the wound for bites in which rabies is considered to be a risk, and for non-bite exposure to animals known or suspected to be rabid.

### 3.3.8 Tetanus

Antitoxin in the treatment of tetanus was introduced in medicine by Behring and Kitasato in 1890. The aim of passive immunisation is to prevent any tetanus toxin produced by *Clostridium tetani* from being fixed to the central nervous system cells since it is very difficult to neutralise the toxin which has already

been fixed to the nervous tissues. Therefore, to be effective, tetanus antitoxin should be administered shortly after injury before any toxin can reach the nervous system. There is still some controversy regarding the place of antitoxin in the treatment of established tetanus.

Heterologous tetanus antiserum is a powerful allergen. Laurent and Parish (1952) estimated an incidence of one death for every 50,000 to 200,000 injections of the antitoxin administered. The incidence of serum sickness following the administration of the heterologous antitoxin has been estimated to range from 5.2% (Moynihan, 1956) to 30% (Scheibel, 1955). Furthermore, in developing countries, more deaths have been caused from anaphylactic shock due to heterologous antitoxin than from tetanus itself (Ericksson and Ullberg-Olsson, 1967). The use of heterologous antitoxin will therefore not be discussed further as it should not be used unless homologous antitoxin is unavailable.

Interest in the use of homologous antitoxin grew following studies by Suri and Rubbo who showed that the use of homologous antitoxin was 100 times more effective than heterologous antitoxin in the prevention of tetanus (Suri & Rubbo, 1961). They also showed that in man homologous antitoxin has a longer half-life. Human tetanus immunoglobulin (TIG) does not cause sensitisation in man and provides a more effective and



longer-lasting immunity than heterologous antiserum.

Passive-active immunisation is recommended for individuals who have never received basic immunisation and who are in need of immediate protection against tetanus. For prophylaxis TIG in a dose of 250 units is administered simultaneously with tetanus toxoid given at a different injection site. In established tetanus, the dose of homologous antitoxin is 5000 to 10000 units. Because tetanus toxin has been found in the cerebrospinal fluid of tetanic patients (Patel and Mehta, 1966), intrathecal administration of antitoxin has been attempted. Most studies (Ildrim, 1975; Diop-Mar et al, 1975 ; Gupta et al, 1980) have shown improved survival among those receiving parenteral and intrathecal antitoxin.

#### 3.3.9 Mumps

There have been many trials of convalescent serum or plasma from mumps patients which have shown a certain degree of benefit or protection against mumps (Rambar, 1946). However, Gellis et al (1945) found that HNIg was ineffective in the prevention or modification of mumps, but that convalescent mumps IgG reduced the incidence of orchitis.

#### 3.3.10 Pertussis

Early investigators used convalescent serum or blood in an attempt to prevent or modify whooping cough.

In 1923, Debre found that convalescent serum was protective if given early during the incubation period. However, the disease was only modified if serum was given late during the incubation period and was unaffected if the serum was administered during the coughing stage (Bradford, 1935).

In 1957, Morris and McDonald found that human hyperimmune pertussis globulin did not prevent pertussis in family contacts. Subsequent trials could not demonstrate any beneficial effect of pertussis IgG in the treatment of pertussis (Bass et al, 1969 ; Balagtas, 1971).

There is no place for pertussis IgG in the treatment or post-exposure prophylaxis of pertussis.

#### 3.3.11 Hypogammaglobulinaemia

Patients with hypogammaglobulinaemia benefit from IgG replacement therapy. The initial trial by the Medical Research Council in 1970 used IM IgG in a weekly dose of 25-50 mg/kg. With the availability of safe IV IgG preparations, it is now possible to administer a much higher dose of IgG, resulting in the achievement of higher serum IgG levels. This is discussed further in section 5.1.5.

#### 3.3.12 Prevention of rhesus (Rh(D)) alloimmunisation

The most common cause of Rh (D) alloimmunisation is feto-maternal bleeding, which may occur at any time

during pregnancy, or following manoeuvres which may cause fetal blood to enter the mother's circulation. The formation of Rh(D) antibodies in Rh(D)-negative mothers may lead to haemolytic disease in any Rh(D)-positive infants born to them.

Stern, Goodman and Berger (1961) showed that if Rh(D) positive red cells were pre-coated with antibody, they did not induce an anti-Rh(D) response when given to Rh(D) negative recipients. This principle was successfully used to prevent maternal sensitisation by fetal red cells (Clarke et al, 1963; Combined study, 1966). It is thought that 20 mg of anti-Rh(D) IgG is required to suppress the immune response for every 1 ml of fetal cells that enter the maternal circulation. The IgG should be administered as soon as possible, as delays in excess of 72 hours reduce the effectiveness of the immune suppression.

Although anti-Rh(D) IgG is very effective, a small number of mothers become sensitised, probably as a result of clinically silent feto-maternal bleeding. Some centres therefore treat all Rh(D) negative mothers with 100 mg of anti-Rh(D) IgG at 28 and 34 weeks of gestation during their first pregnancy (Tovey and Taverner, 1981).

Anti-Rh(D) IgG is also indicated following the accidental transfusion of Rh(D) positive blood to Rh(D) negative women of child-bearing age. The recommended

dose is 20-25 mg/ml of transfused blood by IM route.

Recently, Berlin et al (1985) found that high dose IV HNIg maintained a reduction in anti-Rh(D) antibody level achieved by plasma exchange during pregnancy. They postulated that high dose IgG inhibited maternal antibody synthesis and caused a partial blockade of antibody transport across the placenta.

### 3.4 AREAS OF DEVELOPMENT

The advent of safe IV IgG preparations and of monoclonal antibodies has added to the growing list of accepted clinical uses of immunoglobulins discussed in section 3.3. Specific hyperimmune globulins can now be administered in high doses for the treatment of specific infections. HNIg, however, when used in very high doses, is thought to have immunomodulating properties and consequently high dose IV IgG is increasingly used in clinical conditions that are immunologically mediated. Furthermore, the development of monoclonal antibodies has also opened up new possibilities in the management of malignant conditions. These new developments will be discussed in this section.

#### 3.4.1 Idiopathic thrombocytopenic purpura

During the treatment of children with humoral immunodeficiency with IV IgG, Imbach noticed an increase in platelet count in one such child with coincidental thrombocytopenia. He then treated a mixed group of children with acute and chronic idiopathic thrombocytopenia (ITP) who were refractory to conventional therapy, with IV IgG. Excellent results were obtained in the group with acute ITP, but in the group with chronic disease there was no response in four patients and those who responded initially required maintenance therapy (Imbach et al, 1981). This was

later confirmed by Bussel (1983a ; 1983b).

The mechanism by which high dose IgG leads to an increase in platelet count is unclear. The main postulated modes of action (reviewed by Imbach and Jungi, 1983 ; and Bussel and Hilgartner, 1984) are :

- 1 Reticulo-endothelial system Fc receptor blockade
- 2 Decreased antiplatelet antibody synthesis
- 3 Protection of platelets from antiplatelet antibodies
- 4 Stimulation of platelet production, and
- 5 Clearance of persistent viral infection by infusion of specific antibody.

#### 3.4.1.1 Fc Receptor Blockade

Fc receptor blockade seems to be the most plausible of postulated modes of action. At least two studies have shown a prolonged clearance of antibody-coated autologous red cells, suggesting that there is a slowing of the destruction of antibody-coated platelets. However, reticulo-endothelial system (RES) blockade is short-lived as the rate of red cell clearance returns to the baseline approximately four weeks after IV IgG infusion (Fehr et al, 1982 ; Bussel et al, 1983a) RES blockade, therefore, cannot explain the long-term response to IV IgG.

It also appears that RES blockade is Fc receptor-specific, and that non-Fc mediated phagocytosis is

intact; heat damaged red cell clearance was unchanged in patients after high dose IV IgG (Newland et al, 1983). Similarly, ingestion of antibody coated red cells by circulating monocytes was reduced after IV IgG but ingestion of neuraminidase treated red cells was unchanged (Silberman et al 1984). Finally, Clarkson et al, (1986) successfully treated a patient with refractory immune thrombocytopenic purpura with an anti-Fc receptor antibody.

Possible factors that contribute to Fc receptor blockade are:

- 1 A reduction in the concentration of free macrophage Fc receptors available to bind the Fc part of platelet-associated antibodies
- 2 Modification of affinity of Fc receptors for the Fc part of IgG, (Kimberly et al, 1984)
- 3 Low-grade immune haemolysis of red cells caused by low-titred red cell alloantibodies in the IgG that compete with antibody-coated platelets for available Fc receptors (Salama et al 1983)
- 4 Small amount of IgG aggregates in the IV IgG, contributing to RES blockade.

#### 3.4.1.2 Platelet-Associated Antibody

There is no evidence that high dose IV IgG causes a decrease in platelet-associated antibody as such. A fall normally occurs following improvement

with other forms of treatment for ITP. This decrease is therefore non-specific.

#### 3.4.1.3 Stimulation of Platelet Production

There is also no evidence that IV IgG stimulates platelet production.

#### 3.4.1.4 Protection of Platelets and/or Megakaryocytes from Platelet Antibody

IV IgG does not protect the platelets from the patient's specific platelet antibody. Bussel and Hilgartner (1984) found no increase in immunoglobulin staining on the surface of platelets following IV IgG treatment by direct immunofluorescent staining of fixed platelets using anti-IgG reagents. Furthermore, Blanchette et al (1983) found no difference in the survival of transfused platelets with and without any previous in vitro incubation with IV IgG.

#### 3.4.1.5 Clearance of Persistent Viral Infection by Infusion of Specific Antibody

There is no evidence to substantiate this hypothesis.

#### 3.4.2 Use of IV IgG in other immune cytopenias

Wenske et al (1983), and Newland et al (1984) have successfully used high dose IV IgG without any serious adverse reactions in the treatment of ITP in pregnancy. In addition, there are reports of successful treatment of other kinds of cytopenias such



as post-transfusion purpura (Hamblin et al, 1985 ; Becker et al, 1985), thrombocytopenia secondary to systemic lupus erythematosus, human immunodeficiency virus (Delfraissy et al, 1985). Four patients with post-transfusion purpura benefitted from 0.4 g/kg bodyweight IgG administered intravenously (Becker et al, 1985). Bierling et al (1984) found that high dose IV IgG could prolong the lifespan of incompatible transfused platelets, and thought that they could facilitate the management of leukaemic histocompatibility locus antigen (HLA) immunised patients.

High dose IV IgG has been tried in the treatment of other immune cytopenias. While the use of IV IgG in the treatment of autoimmune neutropenia is well established (Pollack et al, 1982 ; Bussel et al, 1983c) there is some debate regarding its use in the treatment of autoimmune haemolytic anaemia (AIHA). Mueller-Eckhardt et al (1985) have found that IV IgG in a dose of 0.2 g/kg bodyweight daily for five days was ineffective in AIHA, while Bussel et al (1986) found a sustained response in two of three patients treated with very high dose IV IgG (1 g/kg/day) for five to seven consecutive days and maintained on 1 g/kg once every two weeks.

#### 3.4.3 Kawasaki disease

High dose IV IgG administered early in the course of Kawasaki disease seems to reduce the

prevalence of coronary artery abnormalities (Furusho et al, 1984 ; Newburger et al, 1986). Furthermore, in a single case report, IV IgG reduced the duration of fever and arthralgia in a young man with Kawasaki disease (Chavanet et al, 1985).

#### 3.4.4 Myasthenia gravis

Gajdos et al (1984) and Fateh-Moghadam et al (1984), have reported several cases of myasthenia gravis improving after the administration of high dose IV IgG. The improvement was associated with a fall in the acetylcholine receptor antibody level, presumably as a result of reduced antibody synthesis, or of an increased breakdown of total IgG. However, the mode of action remains speculative.

#### 3.4.5 Epilepsy

Laffont et al (1979), and Pechadre et al (1979) have described the effect of empirical therapy for epileptic encephalopathy with gammaglobulin. Ariizumi et al (1984) have reported partial success in the treatment of intractable epilepsy with high dose IV IgG, and Sandstedt et al (1984) have found that high dose IV IgG has benefitted children with therapy-resistant post-encephalitic seizures. The mechanism of action is unclear, but as autoimmune mechanisms can produce epilepsy in animal models, and as children with intractable epilepsy can benefit from immunosuppression,

it is conceivable that high dose IgG can modulate the immune response or may neutralise viruses that may be the cause of encephalopathy.

#### 3.4.6 Malignancy

Wright and Bernstein (1980) have reviewed the use of passively-administered antibody in the treatment against neoplasms. There is evidence that neoplasms in animals as well as in man are at least potentially responsive to treatment with passively-administered antibody, but the response is short-lived. There have been occasional reports of patients with metastatic melanoma who have completely regressed after receiving blood from other patients who have previously undergone complete remission from their melanoma (Sumner et al, 1960). However, subsequent studies have failed to confirm this.

Antibodies raised against malignant cells have been shown to reach their target in bone marrow (Ritz et al, 1981), and in lymph nodes, but the damage to their target cells is usually incomplete and short-lived (Meeker et al, 1985). Certain tumours secrete free antigen (Hellstrom and Hellstrom, 1974) and immune lysis of tumour cells may release large amounts of antigen which may further block the action of antibody. But the main problem with serotherapy of human tumours has been the difficulty in choosing an antigen that is truly

specific, and in obtaining large quantities of antisera of sufficient specificity.

The development of monoclonal antibodies of defined specificity and unlimited quantity has rekindled interest in the use of passively-administered antibody in the treatment of cancer. This is further discussed in section 3.4.9.

Unfortunately, the use of murine monoclonal antibodies may lead to the production of antimouse immunoglobulin antibodies, preventing the usefulness of further administration of murine monoclonal antibodies. Indeed, once an immune response to the foreign antigen has been mounted, further infusions of heterologous antibody do not reach the tumour cells and are associated with toxic reactions (Meeker et al, 1985). But if used in patients with lymphoid malignancies, the immune response is likely to be minimal and clinically insignificant as the patients are immunocompromised. Schroff et al (1985) have found no clinical toxicity related to the immunoglobulin antibody in their patients, and there has been no report of such patients suffering from clinically obvious immune-complex disease. Nonetheless, the use of human monoclonal antibodies would probably avoid this problem (Cosimi et al, 1981).

#### 3.4.7 Rheumatoid arthritis

It is thought that the placental membrane may play an immunomodulating role in patients suffering from active rheumatoid arthritis who undergo remission during pregnancy. Human placental eluted gammaglobulins (PEGG) composed of purified IgG eluted from trophoblastic cells are different from commonly used immunoglobulins or immunoglobulin fractionated from peripheral blood and may have different immunological properties. In a small study, seven of 11 patients with active arthritis improved clinically after PEGG treatment. This was associated with a significant increase in active E-rosette forming cells and in mitogen responsiveness. Such immunomodulating properties were ascribed by the authors to the PEGG treatment (Sany et al, 1982).

#### 3.4.8 Cytomegalovirus (CMV) infection

Infection with CMV is a serious problem following organ transplantation (Rubin et al, 1977 ; Fiala et al, 1975 ; Neiman et al, 1977 ; Rand et al, 1978). These infections can cause a variety of clinical syndromes, some of which may be severe or fatal (Rubin et al, 1977; Fiala et al, 1975; Neiman et al, 1977). CMV infection may also increase the likelihood of graft rejection and the risk of bacterial and fungal superinfection.

Present treatment of severe life-threatening CMV

infection following renal transplantation is unsatisfactory. Antiviral agents such as adenosine arabinoside have not been successful (Rytel and Kauffman, 1976) and although interferon may be of value (Cheeseman et al, 1979), it is unlikely to be generally available. Attempts to prevent CMV by active immunisation have also been unhelpful (Glazer et al, 1979).

Another method for the prophylaxis and treatment of CMV infection is that of passive immunisation. Mouse studies have confirmed the importance of antibody in combating CMV infection by mechanisms which include neutralisation requiring complement and antibody dependent cell mediated cytotoxicity (Araullo et al, 1978; Manischewitz and Quinnan, 1980, and Shanley et al, 1981). Passive immunisation in patients before and after bone marrow transplantation using high-titre specific anti-CMV immunoglobulin or high-titre anti-CMV plasma can prevent or modify CMV infection in these patients (Meyers et al, 1982; Winston et al, 1982).

Passive immunisation for the treatment of CMV infection after renal transplantation has also been attempted. Dijkstra et al (1979) reported the successful use of CMV plasma in a single case of CMV disease after renal transplantation. In an uncontrolled study (Condie et al, 1979) of the efficacy of large doses of HNIg (containing anti-CMV antibodies) given to

patients with life-threatening infections following renal transplantation, it was felt that the treatment benefitted 71% of patients. In those who benefitted, there was a significant increase in antibodies against CMV following IgG administration.

Zaia et al (1979) collected 200 units of plasma with a titre of at least 1:64 against CMV after screening 3080 units. This was fractionated by Cohn's cold ethanol method. The resultant fraction, called CMV IgG, represented a 20-fold increase in CMV titre. Meyers et al (1980b) gave this material to 14 seronegative marrow recipients before and after transplantation, maintaining a complement fixation titre (CFT) of 1:16 in these patients. The treated group and the controls had about the same number of CMV infections (50% and 42% respectively). The authors postulated that the antibody was not effective because the virus was cell-associated at the time of first exposure.

Preliminary data from an uncontrolled study with high-titre specific anti-CMV IgG in patients with life-threatening CMV infection following renal transplantation have shown that anti-CMV IgG may be of value in these patients (Nicholls et al, 1983). Further studies are obviously needed to see if CMV IgG is effective.

### 3.4.9 Monoclonal antibodies

The production of monoclonal antibodies by in vitro techniques (Kohler and Milstein, 1975) has opened up many new avenues for the use of antibodies in clinical medicine (Dick, 1985). Basically, they can be used in imaging lesions, treatment of malignancy and as diagnostic reagents.

#### 3.4.9.1 Imaging

Imaging with radiolabelled antibody has been successful in studies on animals for both malignant and non-malignant lesions. Attempts have been made to delineate myocardial infarcts using antibodies against cardiac myosin, actin or enzymes released from damaged muscles (Haber et al, 1982). But specificity has been difficult to achieve, and there have been problems in producing a clear picture of the extent of the lesion.

Several groups have used labelled mouse monoclonal antibodies for tumour localisation in patients with breast cancer (Rainsbury et al, 1983), colorectal cancer (Farrands et al, 1982), and ovarian cancer (Epenetos et al, 1982). The results of the initial clinical studies were not spectacular. As a diagnostic technique, it is no match for the rapidly advancing computerised radiological techniques such as CT scanning and digital radiology (Sikora et al, 1984). However, it is the prospect of using antibodies as carriers of drugs and



toxins for the efficient localisation of cytotoxic treatment that makes these studies important.

#### 3.4.9.2 Drug Targetting

The use of monoclonal antibodies to target cytotoxic agents aims to increase the amount of drug localising in tumours, particularly metastatic deposits, and also reduce normal tissue toxicity (Baldwin and Byers, 1985). This approach requires that the antibody localises effectively in a tumour and that the drug must be cleaved from the antibody after conjugate binding to a tumour cell, and this usually occurs intracellularly after internalisation of the conjugate (Baldwin, 1986). Drugs selected for antibody conjugation should either be highly cytotoxic, such as ricin (Casellas et al, 1984), or must be amplified to allow sufficient amounts of less intrinsically cytotoxic drugs such as methotrexate to be delivered to the tumour (Baldwin, 1985).

Amplification methods enable more drug to bind to antibody without destroying its reactivity. The drug is linked to a carrier such as human serum albumin and the drug albumin complex is linked to the antibody; at least ten times more drug can be linked to the antibody by this technique (Garnett & Baldwin, 1983).

Ribosomal inhibiting proteins such as ricin are under investigation for use as immunotoxins. Ricin is

composed of two polypeptide chains : B-chain (RTB) binds to galactose residues present on most mammalian cells and facilitates entry into the cell of the A-chain (RTA), which is a potent inhibitor of ribosomal protein synthesis. When RTA is separated from RTB and coupled to antibody, the conjugate is specifically cytotoxic for cells that bind the antibody. RTA conjugates have been produced with several monoclonal antibodies which are highly cytotoxic for human tumour cells (Thorpe, 1984).

#### 3.4.9.3 Unmodified Antibodies for Therapy

In most clinical trials of passive treatment with monoclonal antibodies, the patients were mostly suffering from haematological malignancies including lymphoid solid tumours, which were advanced and had failed to respond to conventional therapy. Nonetheless, remarkable, if short-lived, responses have been achieved, particularly in cutaneous malignancies (Miller and Levy, 1981) and in a few B-cell lymphomas with anti-idiotypic antibody that is a monoclonal antibody directed against the immunoglobulin unique to the malignant clone of B-cells forming the tumour (Stevenson et al, 1984; Meeker et al, 1985).

The Ortho Multicentre Transplant Study Group (1985) found that murine monoclonal antibody OKT3 was significantly better than conventional steroid treatment in reversing acute renal-allograft rejection. The high

incidence of adverse reactions was thought to be due to a physiologic response to mediators released from T-cells after the initial OKT3 treatment.

#### 3.4.9.4 Radiotherapy with Monoclonal Antibodies

The Hammersmith Oncology Group reported treatment of 15 patients with ovarian cancer by intraperitoneal injection of <sup>131</sup>I-labelled monoclonal antibodies; symptoms improved in most patients and complete remissions were achieved in those with stage-III and minimal-residue disease (Epentos, 1985).

#### 3.4.9.5 Bone Marrow Transplantation

Anti-T-lymphocyte monoclonal antibodies are used in man to eliminate mature T-lymphocytes in the graft before transplantation and in vivo to treat graft versus host disease after transplantation. In vitro depletion trials have mainly used two or more complement fixing monoclonal antibodies (Waldman et al, 1984; Prentice et al, 1984), or antibodies conjugated to whole ricin or RTA (Filipovich et al, 1985). Graft versus host disease in HLA-matched sibling transplants can be greatly reduced both in incidence and in severity. However, most studies have reported an increase in graft failure with such T-depleted grafts by comparison with conventional grafts, and it is clear that this will remain important even with HLA-matched grafts (Martin et al, 1985).

Pan-T-lymphocyte monoclonal antibodies have also been used in vivo to treat graft versus host disease with a cocktail of eight complement fixing monoclonal antibodies (Remlinger et al, 1984). There is some indication that therapy produced skin clearing but it was discouragingly ineffective against visceral manifestations.

#### 3.4.10 New areas of development

Another area which requires careful scientific investigation is the definition of the conditions under which synergistic effects between IV IgG and antibiotics may be expected. Dalhoff (1984) observed that, owing to its content in anti-betalactamase antibodies, IV IgG protects B-lactam antibiotics from being hydrolysed, thus resulting in a synergy between acylureido-penicillins and IV IgG.

### 3.5 AREAS OF CONTROVERSY

There is little doubt that passive immunisation will have an increasingly large role to play in clinical medicine, particularly in the prophylaxis and treatment of certain infections and as an immunomodulating agent. In most countries, plasma for fractionation into immunoglobulin preparation is obtained either by separation from conventional blood donations or by plasmapheresis.

HNIG is prepared from plasma pooled from a large number of normal donors and must have a minimum level of antibody against stated viral and bacterial antigens to meet pharmacopoeial standards. However, batches of HNIG prepared from different national or racial groups may vary greatly in their content of specific antibodies. In contrast, specific or high-titre human immunoglobulins are prepared from the plasma of donors who have been screened for high levels of antibody or who have been intentionally immunised to produce high levels of specific antibody prior to plasmapheresis. However, there is some debate as to what constitutes the most suitable source of plasma for the manufacture of hyperimmune globulin.

Intramuscular IgG cannot be given rapidly in large amounts without any discomfort and this limits it as a useful therapeutic agent. As a result, most effort has

been put into developing parenterally administered immunoglobulin preparations. Conventional IM IgG preparations can produce severe acute reactions if given intravenously, due to the presence of complement binding aggregates (Barandun et al, 1962), or vasoactive enzymes (Alving et al, 1980). Manufacturers have modified the plasma fractionation process and also developed several ways of treating the fractionated IgG to avoid reactions, while retaining the biological activity of the molecule. To date, while many such preparations can be safely given by the IV route, there have been doubts regarding the risk of transmitting hepatitis or other viral diseases by IV IgG.

IM IgG manufactured by Cohn's cold ethanol fractionation has an excellent safety record with, to date, no recorded cases of AIDS transmission (WHO Expert Committee : reported by Zuckerman at Symposium on AIDS in Blood Transfusion, 3rd April 1985) and very few reported cases of hepatitis transmission (Petrilli et al, 1977 ; John et al, 1979 ; Tabor and Gerety, 1979). This impressive safety record of IM IgG led to the belief that IgG manufactured by cold ethanol fractionation is inherently safe. However, this has required modification in the light of recent reports of NANB hepatitis transmission by IV IgG products prepared by three different manufacturers (Lever et al, 1984 ; Ochs et al, 1985 ; Weiland et al, 1986). There is,

however, no evidence that the administration of immunoglobulin products by the IV route is inherently infective.

In order to meet the increasing demands for hyperimmune globulins, it is important to identify the most cost-effective means of recruiting plasma donors. This thesis will therefore examine the suitability of random screening for the procurement of CMV, and HB hyperimmune plasma. The effectiveness of random screening will be compared with that of boosting donors with tetanus toxoid for the collection of hyperimmune tetanus plasma.

A new IV IgG preparation is clinically evaluated in the thesis. Its safety and efficacy will be studied with respect to the transmission of hepatitis and the tolerance of high doses of administered IV IgG in order to achieve near normal serum IgG levels in patients with hypogammaglobulinaemia. Finally, an attempt will be made to assess the clinical benefit to those patients following the achievement of near normal serum IgG levels.

4 PLASMA PROCUREMENT



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## 4.1 INTRODUCTION

Plasma collected from blood donors is usually further processed into its various components by ethanol fractionation. It can be obtained either from whole blood donations or by plasma donations (plasmapheresis). Source plasma has two main product use areas; injectable products such as albumin, coagulation factors, and IgG ; and non-injectable products such as laboratory reagents. The source plasma for the manufacture of IgG depends on whether HNIg or specific hyperimmune globulin is the desired end product.

### 4.1.1 Human normal immunoglobulin

HNIg is prepared from pooled plasma collected from at least 1000 randomly-selected healthy blood donors, and the antibody distribution reflects the pattern of infection and immunity in the donor population. Where adequate concentrations of a specific antibody are present in normal IgG (for example, measles antibody), it is unnecessary to prepare a specific high-titred product.

### 4.1.2 Hyperimmune globulin

A hyperimmune globulin may be designated as such if it contains at least five times the antibody potential of the standard preparation per volume. Therefore, for the manufacture of specific hyperimmune globulin, donors possessing suitable levels of specific

antibody are first identified and recruited for plasmapheresis, or they may be intentionally immunised to produce high levels of specific antibody prior to plasmapheresis.

#### 4.1.3 Plasma collection

In 1914, Abel et al described the technique of plasmapheresis in animals and demonstrated that very large amounts of plasma could be withdrawn if the red cells were retransfused. Using this technique, individuals have been able to donate more plasma (Grifole-Lucas, 1952).

Plasma donations can be collected both manually and by machine plasmapheresis. In a manual procedure, whole blood is collected from the donor, and a component separation centrifuge separates off-line the whole blood into red cells and plasma; the red cells are then re-infused back into the donor. This technique is relatively cheap, but is time-consuming and carries the potential risk of returning the wrong red cells to the donor because of the off-line separation. Other potential risks include air embolism and sepsis.

The machine technique harvests source plasma in a closed extracorporeal system (Rock et al, 1981). Plasma separation is achieved by high speed centrifugation with the return of the concentrated red cells to the donor at the end of each cycle. This technique allows the donor



to spend a minimum of time at a maximum of convenience but is expensive, due to the higher cost of the capital equipment and of the disposable software with the unique self-contained red cell/plasma separator chambers, allowing on-line separation of whole blood into red cells and plasma (Gilcher, 1986).

Plasmapheresis by either method is usually undertaken at monthly intervals when around 600 mls (a double donation) of plasma is harvested. Such a loss of plasma in a young healthy donor is replaced within 24 hours by the influx of albumin from the extravascular space. Hepatic synthesis of albumin is subsequently increased so that the deficit is replaced after three to four days (Lundsgaard-Hansen, 1977). Using these data, it is believed safe to limit the volume of plasma collected in one session to 600 mls and to restrict the total plasma volume collected to 15 litres per year. This plasmapheresis programme has been adopted by the International Society of Blood Transfusion. However, certain centres still allow the removal of 1000-1200 ml of plasma per week, and of 50 to 60 litres per year for each donor. But there is continuing debate regarding the long-term safety for the plasma donor following this particular plasmapheresis programme.

The medical criteria for accepting a donor for plasmapheresis are basically those for accepting any blood donor, with the main objectives being the safety,

purity, potency and efficacy of the final product as well as the safety and well being of the donor. Furthermore, if the donor is being recruited for the collection of specific hyperimmune plasma, he/she should possess a high enough level of that specific antibody for which the plasma is collected. Therefore the donor should fulfil the following criteria:

- 1 The possession of a suitable antibody level for the harvesting of specific hyperimmune plasma
- 2 Age less than 50 years
- 3 Bodyweight greater than 50 Kg
- 4 Good venous access
- 5 No major illnesses
- 6 Absence of HBsAg
- 7 Not belonging to a high-risk group for AIDS and absence of markers of infection with Human Immunodeficiency Virus (HIV)
- 8 Availability

#### 4.1.4 Plasma collection for the manufacture of hyperimmune globulin

The need for hyperimmune globulin has been discussed in section 3.3. Basically, the role of hyperimmune globulin lies in the prophylaxis of specific infections, and although its role in the treatment of certain infections has yet to be firmly established, there is increasing evidence to suggest that specific hyperimmune globulin can modify the outcome of certain

infections, particularly in the immunocompromised patient.

This section discusses the problems of recruiting suitable blood donors for the collection of hyperimmune plasma. Regular blood donors who possess suitable levels of useful antibodies are invited to participate in a given programme of plasmapheresis if they fulfil the criteria laid out in section 4.1.3. These donors are usually identified by random screening of the normal blood donor population. The success and cost effectiveness of such screening programmes depend on the prevalence of those particular antibodies in that given donor population, which is itself a reflection of the exposure of the microorganism in the community. Factors that influence the degree of exposure or infection include the infectivity of the microorganism, socioeconomic factors such as level of overcrowding etc,. However, not all infections produce persistently high levels of antibody, and although the population may be immune to the pathogen, only a small number of individuals may possess high enough antibody levels suitable for plasmapheresis.

Where the frequency of donors with a suitable level of an antibody in a given population is low, it may be desirable to enhance the production of such antibodies in selected individuals by immunisation. The success of such an operation depends on the existence of a safe

vaccine that has been shown to produce protective antibodies in vaccinees with a minimum of side effects. If such a vaccine exists, there are theoretical advantages for a planned programme of hyperimmunisation of selected donors for the purpose of producing hyperimmune plasma.

To highlight the relative merits of random screening and of selective hyperimmunisation of blood donors, plasma procurement for the manufacture of CMV, HBV and tetanus hyperimmune globulin has been examined.



## 4.2 PLASMA PROCUREMENT FOR HEPATITIS B IMMUNOGLOBULIN

### 4.2.1 Introduction

There is a clinical need for HB hyperimmune globulin (section 3.2.2). Plasma with high titres of antibody against HBV for the manufacture of HBIG is normally collected from suitable donors who have previously been exposed to HBV infection.

Present plasma procurement systems rely mainly on large-scale screening of the blood donor population to find suitable donors to be recruited for plasmapheresis. Hopkins et al (1981) have found that the prevalence of hepatitis B surface antibody (HBsAb) in the Scottish population is quite low and that screening of the general blood donor population is a large exercise which is costly as well as time-consuming and labour-intensive. Furthermore, the serum level of antibody to HBsAg is not always maintained, leading to a loss of HB immune plasma donors, and as a consequence such screening exercises may have to be repeated at regular intervals. The results of such a screening programme in South East Scotland are analysed here and its effectiveness as a means of identifying high-titre donors suitable for plasmapheresis examined.

### 4.2.2 Materials and Methods

#### 4.2.2.1 Study Design

Between June and October 1983, all blood

donations collected from the normal volunteer blood donor population by the Edinburgh and South East Scotland Regional Blood Transfusion Centre were screened for the presence of HBsAb. HBsAb was subsequently quantified in the positive samples.

Donors with HBsAb levels of 10 IU/ml or greater were identified and approached with a view to recruiting for plasmapheresis. The criteria for suitability for plasmapheresis were :

- 1 Serum HBsAb level of 10 IU/ml or greater
- 2 Age less than 50 years
- 3 Minimum body weight of 50 Kg
- 4 Good venous access
- 5 No major illnesses
- 6 Absence of HBsAg
- 7 Availability

The recruited donors were plasmapheresed at intervals varying between once every four and once every 12 weeks and the volume of each plasma donation was recorded by weighing. Before each plasmapheresis, quantitation of HBsAb level was carried out and liver function tests and absence of HBsAg were monitored.

#### 4.2.2.2 Biochemical Assessment

Venous blood samples were collected at each plasmapheresis for liver function tests performed by the Clinical Chemistry Laboratory, Royal Infirmary of

Edinburgh, by Multiple Analysis with Computer System (SMAC 2).

#### 4.2.2.3 Screening and Quantitation of HBsAb level

Screening of the blood donations was carried out using a solid-phase sandwich immunoassay adjusted to detect samples with HBs antibody level above 1 IU/ml (Hopkins et al, 1981). The antibody level in positive sera was measured using AUSAB (Abbott Laboratories, Chicago, USA) radioimmunoassay. A laboratory standard, calibrated against a WHO standard obtained from the Amsterdam Red Cross Central Laboratory, was diluted in 0.1 M Tris/HCl pH 7.5 containing 50% normal human serum (HBsAg/anti-HBs negative) to values of 0.32, 0.16, 0.08, 0.04, 0.02 and 0.01 IU/ml. Anti-HBs containing sera were diluted 1 in 10, 1 in 100, 1 in 1000 and 1 in 10,000. Count rates were automatically processed by a NE 1612 gamma counter (Nuclear Enterprises, Edinburgh, Scotland) programmed to construct a standard curve against which to compare appropriate test serum dilutions.

#### 4.2.2.4 Assessment of Hepatitis B Status

Venous blood samples were assayed for HBsAg by radioimmunoassay (Blood Products Laboratory, Elstree, U.K.).

#### 4.2.3 Results

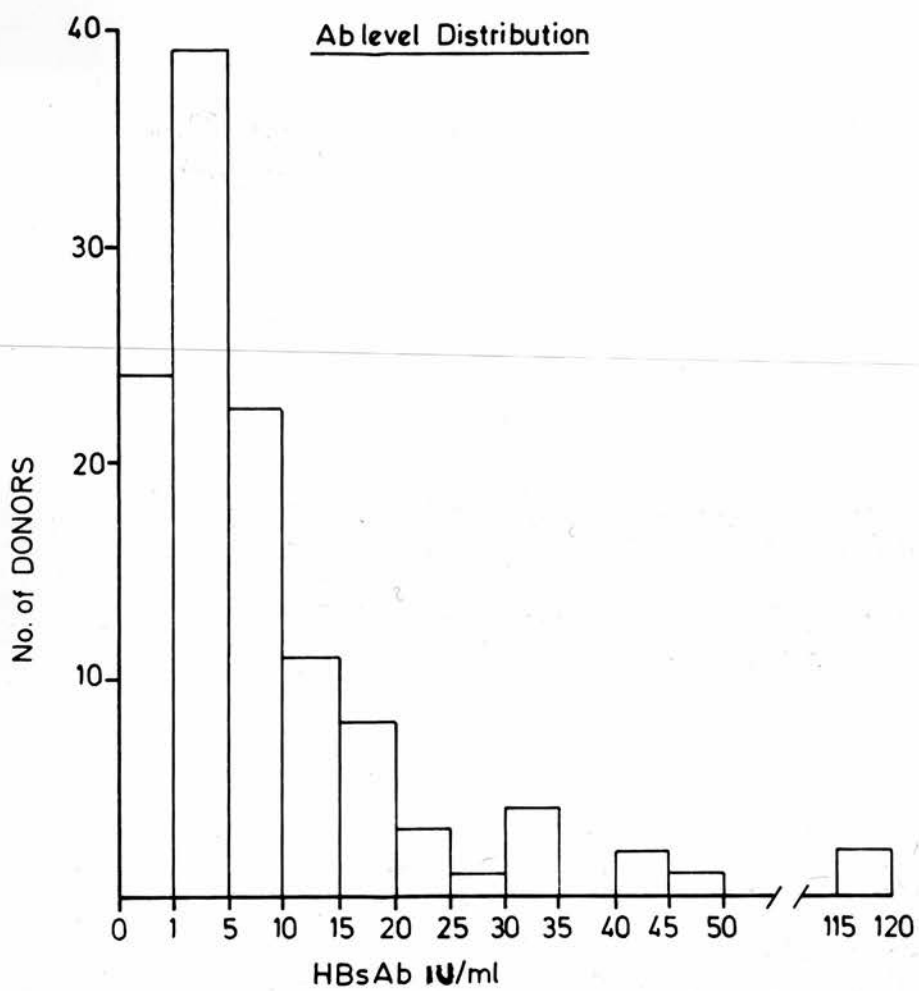
Of the 29,265 serum samples screened between June and October 1983, 129 samples contained more than 1 IU/ml HBsAb. Subsequent quantitation of these 129 positive serum samples showed the following distribution (see fig 4.2.1).

A total of 19 donors had HBsAb levels of 10 IU/ml or greater with a range of 10.0 to 116.0 (mean 15.2 IU/ml). Of these 19 newly identified donors that were approached, 10 agreed to be recruited for regular plasmapheresis. One donor had to be withdrawn because routine liver function tests showed evidence of chronic liver disease. Twelve months after the screening programme only two of the newly-recruited nine were still donating hyperimmune HB plasma. Of those lost, five moved elsewhere and in two cases the HBsAb level had fallen and remained consistently below 10 IU/ml.

No donor developed abnormal ALT values during the study.

##### 4.2.3.1 Quantity of Plasma Collected

A total of 30.5 litres of hyperimmune HB plasma was collected from the nine newly-recruited donors over the 12 months following recruitment (0.8 to 6.5 litres per donor). The mean volume of plasma per donor collected was 3.4 litres. A total of 424,380 IU of HBsAb was collected with a mean plasma concentration of



**Figure 4.2.1**  
**HBsAb level distribution**

13.9 IU/ml of HBsAb.

#### 4.2.4 Discussion

HBIG is used in the prevention of HBV infection following accidental exposure to the virus and in infants born to mothers who are chronic carriers of the virus. The requirement for this immunoglobulin is likely to continue or increase in view of the increasing incidence of HBV infection (Communicable Diseases, Scotland, 1984) despite the availability of a safe HBV vaccine. As a result, a low cost radioimmunoassay for the screening of donors has been developed (Hopkins et al, 1981) and the long term value of random screening of donors for high titre HBsAb plasma was analysed. Of all the donors identified following the screening of 29,265 blood donations, only two were still donating regularly 12 months after they had been recruited.

The experience in South East Scotland in recent years is that every year on average a third of donors from the HBsAb plasmapheresis panel become unsuitable because of falling antibody level, or because they have moved elsewhere. Therefore, to maintain a constant panel of say 15 donors it would be necessary to repeat the screening programme at less than three-yearly intervals. This present system therefore appears to be a very inefficient way of identifying and recruiting hepatitis B plasma donors.

In previous years, five other screening campaigns have been carried out in Edinburgh ; the very poor recruitment rate is confirmed (Table 4.2.1).

It is therefore important that other methods for procuring HBsAb plasma for the manufacture of HBIg be examined. In particular, now that a safe HBV vaccine is available, the immunisation of suitable donors should be considered. However, while it is generally very difficult for individuals to achieve high enough antibody levels suitable for plasmapheresis (greater than 10 IU/ml) following a primary course of immunisation with HBV vaccine (Szmuness et al, 1981b; Dienstag et al, 1984), preliminary studies in the North London Blood Transfusion Service have demonstrated that high enough antibody levels can be reached in individuals who have pre-existing naturally-acquired HBs antibody by boosting. A consistent 10-fold rise and in certain cases a 60-fold rise in antibody levels following boosting with the Merck, Sharp & Dohme HBV vaccine has been demonstrated (Barbara et al, 1983). It is therefore suggested that boosting individuals with pre-existing naturally-acquired HBsAb be examined as a means of recruiting suitable HB immune plasma donors.

Date of screening	No. of donations screened	No. (%) of positive donations		No. (%) suitable for plasmapheresis		No. recruited for plasmapheresis
		n	%	n	%	
May 1980 to Aug 1980	10,095	29	0.28	0		0
Oct 1980 to Dec 1980	11,849	47	0.39	3	0.025	1
Feb 1981 to May 1981	16,231	58	0.35	4	0.025	0
May 1981 to Sep 1981	3,210	10	0.31	1	0.031	1
Sep 1981 to Aug 1982	28,384	46	0.16	8	0.028	5
June 1983 to Oct 1983	29,265	129	0.67	26	0.088	9

Table 4.2.1 Screening campaigns carried out in Edinburgh to detect HBsAb positive donations



## 4.3 PLASMA PROCUREMENT FOR CMV HYPERIMMUNE GLOBULIN

### 4.3.1 Introduction

CMV infection is an important cause of morbidity and mortality in organ and bone marrow transplant recipients (Nieman et al, 1977; Peterson et al, 1980). In order to reduce the frequency and severity of CMV infection in such patients, a variety of strategies including the prophylactic administration of CMV hyperimmune globulin have been proposed. The efficacy of CMV hyperimmune globulin in the prophylaxis and treatment of CMV infection in this setting has been discussed in section 3.4.10.

The demand for CMV hyperimmune globulin in these clinical conditions is likely to increase with the increasing number of transplants performed. Source plasma for the production of CMV hyperimmune globulin is collected from donors with high levels of naturally occurring CMV antibodies. These donors are identified by random screening of the donor population using a number of serological tests.

The results of a screening programme for indentifying such donors are described, and the effectiveness of the programme discussed.

### 4.3.2 Study design

During a seven-week period, from 27 October 1982 to 16 December 1982, 845 random donors visiting the

Blood Donor Centre in Edinburgh were screened for the presence of high-titre CMV antibody by enzyme-linked immunosorbent (ELISA) test. The screening was performed by Dr GR Barclay and his technicians at the Edinburgh Blood Transfusion Centre.

Donors who fulfilled the following criteria were later invited for regular plasmapheresis;

- 1 ELISA value equal to or greater than that found for a CMV-positive plasma previously reported to have a titre of 1 in 40 by complement fixation test (CFT) (PHLS, Colindale Hospital, London) or 1 in 32 (Department of Virology, University of Edinburgh).
- 2 CFT titre of 1 in 16 or greater (Department of Virology, University of Edinburgh)
- 3 Good venous access
- 4 No major illnesses
- 5 Absence of HBsAg
- 6 Age less than 50 years
- 7 Availability

#### 4.3.3 Results

Of the 845 donors screened, 85 (10%) were considered suitable by serological criteria using ELISA. After further testing by the Department of Virology at Edinburgh University using CFT, 74 donors were contacted for possible recruitment.

#### 4.3.3.1 Response Rate

- (a) 11 donors did not respond to the request letter
- (b) 13 donors were considered unsuitable for plasmapheresis on medical grounds
- (c) Two donors were not contacted due to clerical mishap
- (d) 48 donors were considered suitable and agreed to be regularly plasmapheresed.

#### 4.3.3.2 Follow-up

The number of donors who remained on the plasmapheresis panel at 6, 9, 12, 24, and 36 months is shown in Table 4.3.1. Donors were withdrawn from plasmapheresis for a variety of reasons as shown in Table 4.3.1. The single most common one was a fall in serum CMV antibody level; 17 donors were withdrawn from the panel within the first year for that reason. Fourteen donors were withdrawn for medical reasons; abnormal liver function tests (five donors), anaemia (three donors), and the development of antibodies to HB core antigen.

A total of 568 single donations equivalent to 142 litres of high-titred CMV plasma were collected within the first year.

Only 25% of donors recruited from the screening programme were still donating at 12 months, and this figure fell to 18.75% and 12.5% at 24 and 36 months.

Time from recruitment (months)	6	9	12	24	36
No. donors active	36	25	12	9	6
No. donors withdrawn because of					
Low titre	4	12	17	18	20
Medical condition	4	6	14	15	16
Lost to follow-up	3	3	3	3	3
Moved away from centre	1	2	2	3	3
Total No. withdrawn	12	23	36	39	42

Table 4.3.1    Distribution of donors following recruitment : the medical reasons included anaemia, pregnancy and liver disease

#### 4.3.4 Discussion

The frequency of CMV infection in adults varies between and within countries, probably depending on socioeconomic and hygienic conditions. The infection, usually asymptomatic, is followed by an antibody response. Children of low socioeconomic status acquire the infection earlier than whites (Li & Hanshaw, 1966; Monif et al, 1970). In the study by Szmuness et al (1975), male volunteer blood donors who were mostly white and middle class displayed a relatively low prevalence of CMV-CFT antibody. The prevalence of CMV-CFT antibody is also thought to rise with age (Krech et al, 1973 ; Kane et al, 1975 ; Monif et al, 1974).

In this study, 10% of randomly selected donors had CMV antibody levels suitable for recruitment into a regular plasmapheresis programme. In contrast, Zaia et al (1979) found that source plasma for the production of hyperimmune CMV IgG could be obtained from 5% of the normal blood donor population. The marked decrease (75%) in the number of donors who remain suitable for plasmapheresis after one year suggests that this form of recruitment is relatively inefficient. Of the original 48 donors, 17 (35%) were withdrawn because of falling antibody levels. Interestingly, Waner et al (1973) found marked fluctuations in CMV antibody levels in 19 of 20 plasmapheresed donors.

In view of the relatively low prevalence of donors with suitable CMV antibody levels in contrast to the high prevalence of high CMV antibody in the homosexual population (50%), Ikram et al (1983) have suggested that an adequate supply of hyperimmune CMV plasma could be obtained from the homosexual population. However, in the light of the present AIDS epidemic, this plasma source is totally unacceptable.

However, data from this study show that 44% of female donors, compared to 21% of male donors, dropped their CMV antibody level below that acceptable for plasmapheresis within 12 months of recruitment. Thus, male donors would appear more suitable for plasmapheresis.

While random screening of blood donors is inefficient and requires to be frequently repeated to meet the increasing demands for CMV IgG, the situation will remain unchanged until a reliable safe vaccine becomes available. To date hyperimmunisation of donors with a CMV vaccine is not feasible as there is still concern over the long-term safety of the currently available CMV vaccines, particularly with respect to their potential oncogenicity. Several herpes viruses have been associated with cancer (natural and experimental) and CMV has been shown to cause morphologic and neoplastic transformation (Albrecht and Rapp, 1973). Random screening of blood donors therefore

remains the only means of identifying CMV hyperimmune plasma donors.

#### 4.4 HYPERIMMUNE TETANUS PLASMA PROCUREMENT

##### 4.4.1 Introduction

Tetanus immune globulin (TIG) is manufactured from plasma collected from human donors who have high levels of tetanus antibody. These donors are usually selected shortly after a course of tetanus immunisation or a tetanus boost following trauma. However, this method is not entirely satisfactory because not all donors achieve suitably high antibody levels (greater than 10 IU/ml) after immunisation. Some plasma collecting organisations therefore boost selected donors to render them suitable for donating hyperimmune tetanus plasma (Cook et al, 1976; Rubin et al, 1980).

Local and mild systemic reactions following tetanus toxoid administration are quite common (Deacon et al, 1982), and the frequency and severity of such reactions is thought to increase with the number of vaccine doses administered (White et al, 1973). Boosting of blood donors with tetanus toxoid would therefore have to take into account the level of adverse reactions that would be acceptable to the donor population, as well as the problem of compensation to the donor in the event of these reactions being unduly serious.

Adverse reactions are thought to be due to hypersensitivity to the toxoid or to proteins of *Clostridium tetani* which co-purify with toxoid during



the precipitation process used in the conventional preparation of toxoid. It was therefore thought possible that a purer toxoid might be less reactive, and thus safer for hyperimmunisation of donors.

Such an immune purified toxoid containing fewer contaminant proteins has been prepared by affinity chromatography at Wellcome Research Laboratories. A study was therefore designed (1) to compare the IPT toxoid with conventional toxoid in terms of tetanus antibody response and adverse reactions in adult vaccinees, and (2) to define the characteristics of the donor most suitable for a programme of hyperimmunisation with tetanus toxoid; and in particular, to examine the factors that influence the incidence of adverse reactions to tetanus boosting, and those that determine the persistence of antibody levels following boosting.

#### 4.4.2 Volunteers, Materials and Methods

##### 4.4.2.1 Volunteers

Two hundred and five healthy adult volunteers of both sexes aged 18 - 64 years were recruited from blood donors attending the SNBTS, Edinburgh.

Exclusions from the study were:

- 1 Administration of TIG within six weeks prior to the study
- 2 Administration of tetanus toxoid within 12 months prior to the study

### 3 Pregnancy

- 4 Subjects who experienced a severe reaction to a previous dose of tetanus vaccine.

#### 4.4.2.2 Vaccines

CPT (Adsorbed tetanus vaccine, Wellcome lot IPPT 31) contained 10 Lf of tetanus toxoid (1770 Lf/mg protein N) and had an immunising potency of 80.2 IU/dose. IPT (Wellcome lot IPPT 27) contained 10 Lf of tetanus toxoid (2317 Lf/mg protein N) and had an equivalent immunising potency (109.4 IU/ dose). IPT was purified by antibody affinity chromatography using equine tetanus antibody conjugated to Sepharose by the cyanogen bromide method. Both vaccines contained 0.69 mg Al +++ / dose.

#### 4.4.2.3 Methods

Vaccination and reaction histories were determined by interview. Initial venous blood samples were provided for assessment of tetanus antibody. Volunteers were matched for age and sex (Table 4.4.1), and with six exceptions tetanus antibody titres were randomly assigned to receive CPT (n = 102) or IPT (n = 103). A single 0.5 ml dose of vaccine was administered to each volunteer by deep subcutaneous injection using a needle and syringe. All doses were selected by reference to a list assigning volunteer names to treatment numbers, and were therefore

	CPT	IPT
No. in group (% males)	102 (50%)	103 (51%)
Mean age (range) in years	34 (18-64)	32 (18-64)

Table 4.4.1      Characteristics of recipients of conventionally purified tetanus (CPT) and immune purified tetanus (IPT) toxoid vaccines

administered on a double-blind basis.

After the completion of the vaccine trial, donors were invited to be plasmapheresed if they fulfilled the following criteria :

- 1 Serum antibody level of 10 IU/ml or greater
- 2 Age less than 50 years
- 3 Body weight greater than 50 kg
- 4 Good venous access
- 5 No major illnesses
- 6 Absence of HBsAg
- 7 Availability

Thirty-six donors were invited to donate plasma for the manufacture of TIG. The frequency of plasmapheresis varied from once every four weeks to once every 12 weeks, depending on availability. Donors whose tetanus antibody levels dropped below 10 IU/ml on two successive occasions were withdrawn from the plasmapheresis panel.

All volunteers provided informed consent to participation in the study. The protocol was approved by the Ethical Committee of the SNBTS.

#### 4.4.2.4 Statistics

Statistical evaluation of significance of differences in frequencies of events between groups was determined by Fischer's exact test.

#### 4.4.2.5 Clinical Reactions

Vaccinees were asked to complete a daily record for seven days after vaccination so as to record any reactions at the site of injection (pain, redness, tenderness and the presence of nodule), as well as systemic reactions (headache, feverishness and malaise). Pain, redness and tenderness were graded 1-3 according to whether they were considered mild, moderate or severe. Volunteers recorded their morning oral temperature daily for seven days after immunisation.

#### 4.4.2.6 Measurement of Tetanus Antibody

Blood was collected from 204 volunteers before and approximately two weeks after immunisation. One recipient of IPT failed to provide paired serum samples. The concentration of circulating tetanus antibody was determined on paired pre- and post-immunisation sera by ELISA for specific IgG antibodies (Dr GR Barclay, unpublished). The concentration of tetanus antibody in a subset of vaccinees was also measured by the scientific staff at the Wellcome Research Laboratories using the toxin neutralising test of Glenny and Stevens (1938).

#### 4.4.3 Results of vaccine trial

The groups were balanced for age, sex and preimmunisation tetanus antibody titres. (Table 4.4.1).

#### 4.4.3.1 Clinical Reactions

Calendar records were returned by 204 participants in the study ; one recipient of immune purified vaccine failed to return a reaction card.

Inspection of the calendar records revealed no significant reaction to either vaccine. Only transient mild systemic symptoms and signs with mild discomfort at the site of injection was recorded. No significant difference in the overall frequency of reactions was detected between the groups, with 83/102 (81%) of CPT recipients as compared to 88/102 (86%) of IPT recipients recording any reactions during the study ( $p > 0.2$ ).

The frequency of local reactions, defined as redness, pain, tenderness and the presence of a lump at the injection site, are shown in Table 4.4.2. The frequency of local reactions reported in the recipients of CPT was 76/102 (75%) as compared to 83/102 (81%) in the group receiving IPT. The difference is not statistically significant. Further details of the incidence of local reactions are shown in Table 4.4.2.

In the seven days following vaccination, 29 (28%) CPT recipients and 29 (28%) IPT recipients experienced systemic symptoms or signs that might have been associated with the vaccines (Table 4.4.3). These included pyrexia (temperature  $> 37.5$  C), headache, influenza-like symptoms, nausea, dizziness, malaise, myalgia, or peripheral paraesthesia. Other symptoms

Vaccine	CPT	IPT
No. of participants	102	102
Any local reactions		
No. (%) reporting	76 (75)	83 (81)
Mean no. of days* (range)	4.14 (1-7)	4.17 (1-7)
Redness		
No. (%) reporting	54 (53)	53 (52)
No. (%) severe reactions	5 (5)	5 (5)
Mean no. of days* (range)	3.30 (1-7)	3.57 (1-7)
Pain		
No. (%) reporting	30 (29)	29 (28)
No. (%) severe reactions	0 (0)	1 (1)
Mean no. of days* (range)	2.47 (1-5)	2.28 (1-7)
Tenderness		
No. (%) reporting	62 (61)	61 (60)
No. (%) severe reactions	5 (5)	1 (1)
Mean no. of days* (range)	2.95 (1-7)	2.85 (1-7)
Lump at injection site		
No. (%) reporting	49 (48)	53 (52)
Mean no. of days* (range)	4.41 (1-7)	4.30 (1-7)
Other reports**		
No. (%) reporting	23 (23)	18 (18)

\* Total no. of person days on which reactions were reported

\*\* Itch; bruise; swelling

Table 4.4.2 Local reactions reported by participants in a clinical comparison of conventionally purified tetanus (CPT) and immune purified tetanus (IPT) toxoid

Vaccine	CPT	IPT
Any systemic reactions		
No. (%) reporting	29 (28)	29 (28)
Mean no. of days* (range)	2.28 (1-7)	1.93 (1-7)
Pyrexia (>37.5 C)		
No. (%) reporting	5 (5)	5 (5)
Mean no. of days* (range)	1.80 (1-4)	1.50 (1-2)
Headache		
No. (%) reporting	14 (14)	14 (14)
Mean no. of days* (range)	2.36 (1-6)	1.64 (1-7)
Feverishness		
No. (%) reporting	4 (4)	6 (6)
Mean no. of days* (range)	2.00 (1-3)	1.83 (1-3)
Miscellaneous reports †		
No. (%) reporting	13 (13)	12 (12)
Mean no. of days* (range)	2.1 (1-6)	1.4 (1-3)
Other reactions ‡		
No. (%) reporting	5 (5)	7 (7)
Mean no. of days* (range)	2.0 (1-3)	2.1 (1-7)

\* Total no. of person days on which reactions were reported.

† Possible vaccine-associated reactions: nausea, dizziness,

‡ malaise, peripheral paraesthesia, diarrhoea, abdominal pain, urinary frequency, upper respiratory infections.

Table 4.4.3 Systemic reactions reported by participants in a clinical comparison of conventionally purified tetanus (CPT) and immune purified tetanus (IPT) toxoid



such as diarrhoea, abdominal pain, urinary frequency and sore throats may have been related to concurrent infections, and were therefore categorised separately. One recipient of CPT reported peripheral paraesthesia on the second and third post- vaccination days. It is unclear whether this arose in the injected or contralateral arm. Analysis of the frequency of symptoms and signs did not demonstrate any statistically significant difference between the two vaccine groups.

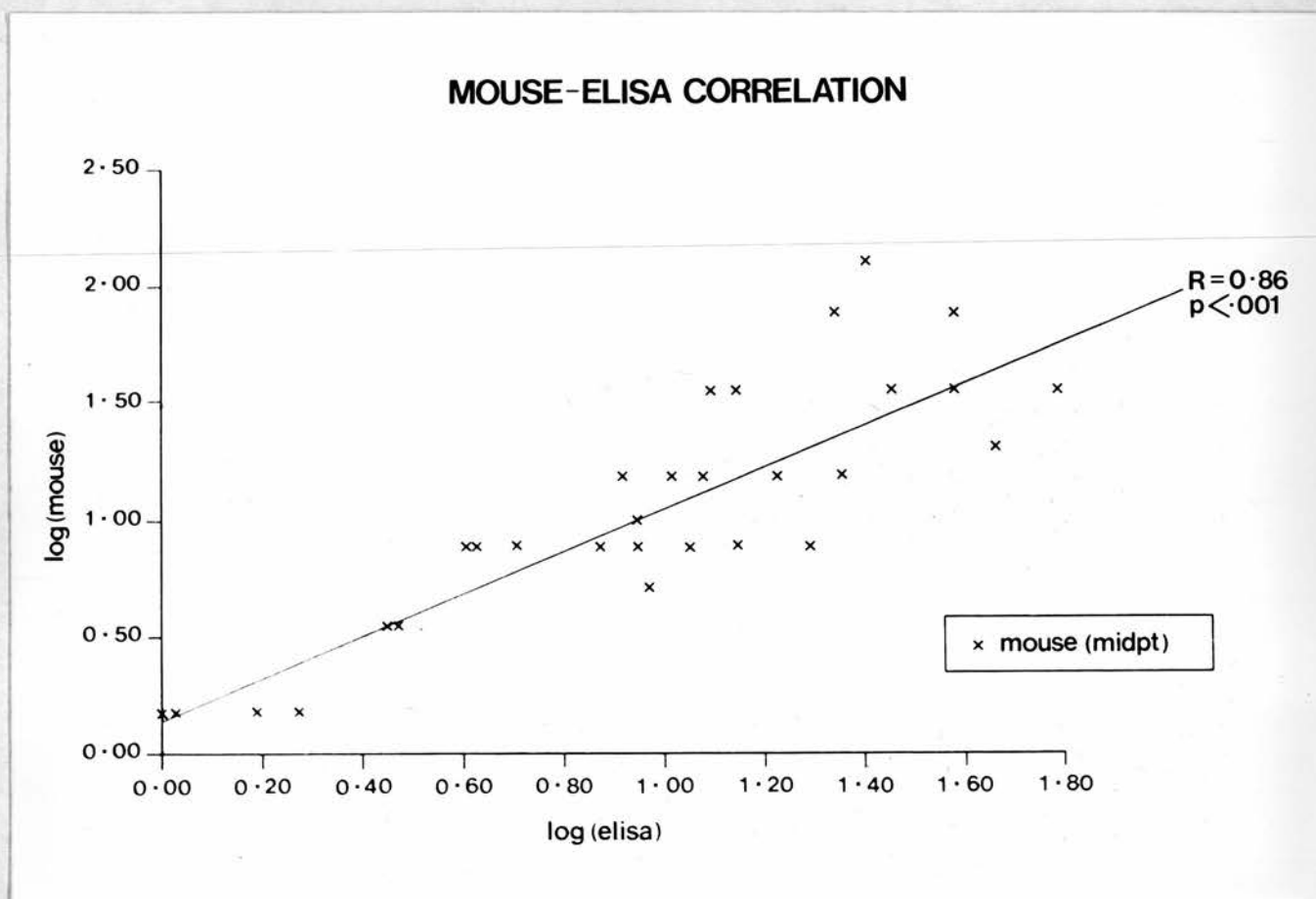
#### 4.4.3.2 Vailidity of ELISA Method

Figure 4.4.1 shows the correlation between the ELISA and the mouse neutralisation test for the measurement of tetanus antibody ;  $r = 0.86$ .

$$\log (\text{mouse}) = 0.13 + 0.93 \log (\text{ELISA})$$

#### 4.4.3.3 Antibody Response

Increases in tetanus antibody in the recipients of each vaccine, assessed by ELISA, are shown in Tables 4.4.4 and 4.4.5. Forty-four (75%) of 59 recipients of CPT, and 45 (67%) of 59 recipients of IPT with previously undetectable antibody levels developed concentrations indicative of immunity to tetanus, i.e., at least 0.01 IU/ml. Altogether, 83 CPT recipients (81%) and 86 IPT recipients (84%) had an increase in tetanus antibody following immunisation. There was also no difference in the frequency and magnitude of antibody response in the two groups.



**Figure 4.4.1**

Correlation between tetanus antibody measurements  
by ELISA and mouse neutralisation test

	CPT	IPT
Total no. assessed	102	102
Pre-immunisation tetanus antitoxin value = 0	59	59
Post-immunisation tetanus antitoxin value = 0	15	14
No. with an increase in tetanus antitoxin	83	86
GMT* pre-immunisation	1.48	1.52
post-immunisation	6.21	6.65

\*GMT = Geometric mean tetanus antitoxin titre calculated on the assumption that antitoxin titres were below the limits of detection, i.e. 0 taken to equal the lowest possible titre.

Table 4.4.4 Serological responses to conventionally purified tetanus (CPT) and immune purified tetanus (IPT) toxoid as assessed by ELISA

	CPT	IPT
> 2.5 IU/ml	12	15
2.5-5.0 IU/ml	7	15
5.0-10.0 IU/ml	29	23
10.0-20.0 IU/ml	23	20
20.0-40.0 IU/ml	12	11
40.0-80.0 IU/ml	0	2
Total	83	86

Table 4.4.5    Increase in tetanus antitoxin.  
 Analysis of increase in tetanus antitoxin  
 measured by ELISA.  
 CPT = conventionally purified tetanus  
           toxoid  
 IPT = immune purified tetanus toxoid

#### 4.4.4 Data following boosting

The two vaccines were found to be similar with respect to the frequency of reactions encountered in the vaccinees and to their immunogenicity. For the purposes of data presentation and analysis, no distinction was made between the two vaccines.

##### 4.4.4.1 Effect of Age, Sex and Preimmunisation Tetanus Antibody Level on Antibody Level at Two Weeks after Immunisation

There was a significant correlation between age and antibody level assayed two weeks after immunisation ( $r = -0.30$ ;  $p < 0.001$ ), and between age and increase in antibody level defined as the difference between post- and preimmunisation antibody levels ( $r = -0.24$ ;  $p < 0.001$ ) (Table 4.4.6).

There was no significant sex difference in the magnitude of antibody increase (Table 4.4.7).

The magnitude of antibody increase was independent of the preimmunisation antibody level (Table 4.4.8).

##### 4.4.4.2 Effect of Preimmunisation Antibody and Age on Incidence of Reactions to Vaccine

The sum of the number of days on which the vaccinees experienced any moderate or severe symptoms was used for the analysis of adverse reactions. There was no significant correlation between preimmunisation antibody level and overall reactions; however, the relationship suggested that there may be an unusually

Final antibody level, IU/ml	Age, years				
	<25	25-35	25-50	>50	19-65
0	1 (2)	10 (13)	15 (30)	5 (24)	31
<10	28 (49)	32 (42)	18 (36)	10 (48)	88
>10	28 (49)	34 (45)	17 (34)	6 (29)	85
Total	57 (100)	76 (100)	50 (100)	21 (100)	204

Figures in parentheses indicate percentage.

Table 4.4.6 Relationship between age and antibody response

Final antibody level, IU/ml	Male	Female	
0	14 (13%)	17 (17%)	31
<10	45 (43%)	43 (43%)	88
>10	46 (44%)	39 (39%)	85
Total	105 (100%)	99 (100%)	204

Table 4.4.7 Relationship between sex and antibody response

	Preimmunisation antibody level, IU/ml				
	0	0-2	2-4	4-8	>8
Total Number	118	33	23	21	9
No. with increase in antibody level post-immunisation	89(75)	31(94)	22(96)	20(95)	7(78)
Increase in anti- body, IU/ml					
Mean	8.76	8.44	9.50	8.08	7.75
$\pm$ SD	10.60	7.21	6.46	8.87	10.44

Figures in parentheses indicate percentage.

Table 4.4.8 - Relationship between preimmunisation antibody level and antibody increase



high incidence of reactions amongst vaccinees with antibody levels which were detectable but less than 2.0 IU/ml (Table 4.4.9). Males had significantly fewer reactions than females ( $p < 0.01$ ) (Table 4.4.10), but there was no evidence that age had any effect on the incidence of adverse reactions.

When the individual reactions were analysed, there was a significantly higher incidence of moderate or severe tenderness and redness among the females ( $p < 0.01$ ; Table 4.4.10). There was also a correlation between preimmunisation antibody level and pain ( $r = 0.21$ ;  $p < 0.01$ ) and between preimmunisation antibody level and frequency of tenderness ( $r = 0.20$ ;  $p < 0.01$ ). The incidence of individual reactions was also analysed in vaccinees grouped according to their preimmunisation antibody levels. While there was a significantly higher incidence of moderate and severe pain at the injection site in subjects with preimmunisation antibody levels above 8 IU/ml ( $\chi^2 = 4.15$ ;  $p < 0.05$ ), the increased incidence of moderate and severe tenderness in that group was not statistically significant ( $\chi^2 = 3.80$ ;  $p < 0.05$ ), when compared with the group with no detectable preimmunisation antibody level.

Interestingly, the group with detectable preimmunisation antibody levels of less than 2 IU/ml showed the highest frequency of reactions, which in many cases were significant as compared to the group without

	Preimmunisation antibody level, IU/ml				
	0	0-2	2-4	4-8	>8
Total number	118	33	23	21	9
Local reactions					
Redness (%)	17(14)	9(27)	5(22)	3(14)	2(22)
Pain (%)	3(2)	1(3)	2(9)	3(14)	2(22)*
Tenderness (%)	9(8)	11(33)**	1(4)	5(24)	3(33)
Lump (%)	55(47)	17(52)	12(52)	13(62)	6(66)
Systemic reactions					
Temperature (%)	4(3)	4(12)	1(4)	1(5)	0
Headache (%)	13(11)	9(27)*	3(13)	2(10)	2(22)
Feverishness (%)	2(2)	4(12)	1(4)	2(10)	1(11)
No moderate or severe reaction	95(81)	14(42)	19(83)	13(62)	5(56)
Some moderate or severe reaction	23(30)	19(59)	4(17)	8(38)	4(44)

Figures in parentheses indicate percentage

\*  $0.05 > p > 0.025$ ; \*\*  $p < 0.001$

Table 4.4.9 Correlation between preimmunisation antibody level and reaction to vaccine

	Male n = 105	Female n = 99
Local reactions		
Redness (%)	11 (10%)	25 (25%)*
Pain (%)	3 (3%)	8 (8%)
Tenderness (%)	8 (8%)	21 (21%)*
Lump (%)	46 (44%)	57 (58%)
Systemic reactions		
Temperature (%)	2 (2%)	8 (8%)
Headache (%)	15 (14%)	14 (14%)
Fever (%)	3 (3%)	7 (7%)
Total reactions (pain, tenderness, fever, headache)	22 (21%)	36 (36%)

\*  $p < 0.01$

Table 4.4.10 Frequency of reactions among sexes

detectable preimmunisation antibody.

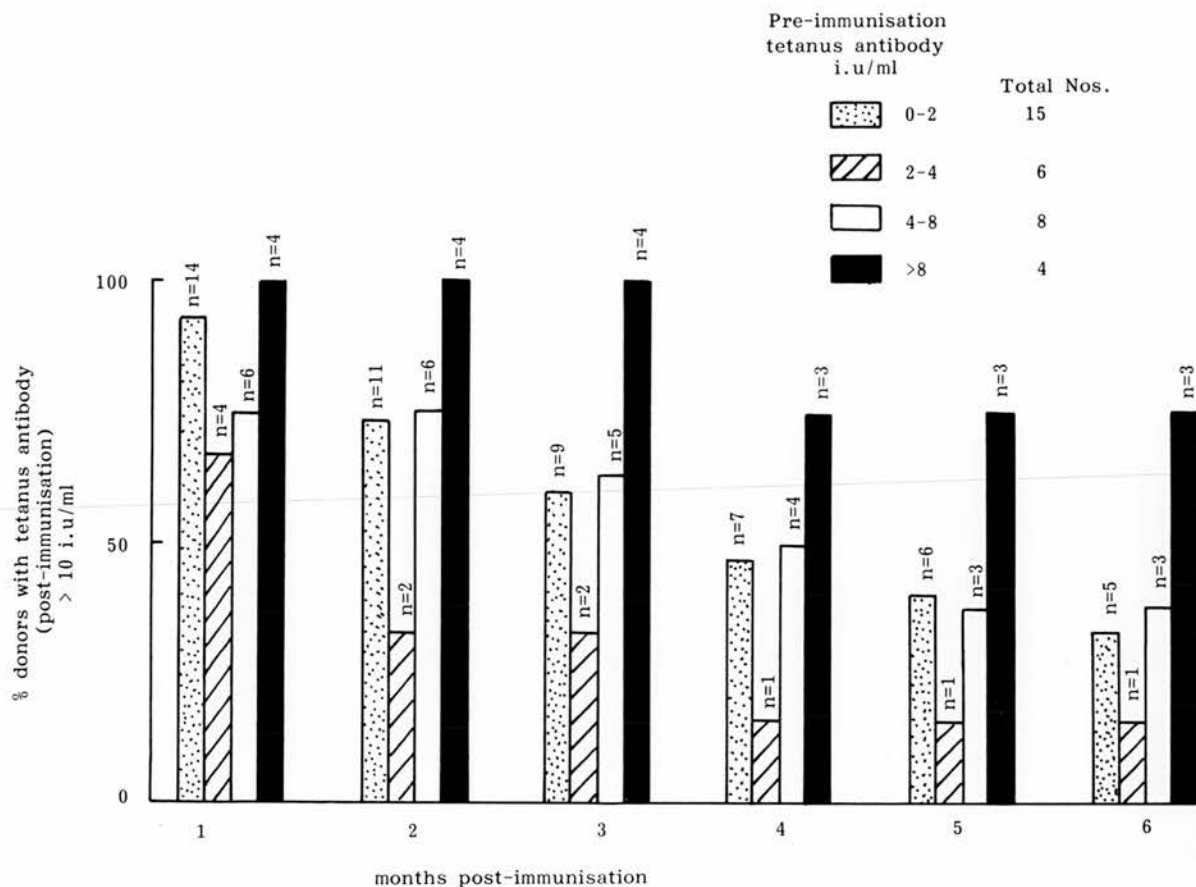
#### 4.4.4.3 Volume of Hyperimmune Plasma Collected

Thirty-six donors (23 men and 13 women) with high post-immunisation antibody levels were recruited for plasmapheresis. Three donors were withdrawn from plasmapheresis: two men were unavailable to attend regularly because of business commitments, and one woman developed a medical condition unrelated to immunisation or plasmapheresis. By six months after immunisation, plasmapheresis had been stopped in 21 of the 33 remaining donors (62%) because their antibody levels had fallen below 10 IU/ml. The volume of plasma collected in the six months following immunisation was 54.5 litres containing a total of 1,201,400 IU of tetanus antibody with a mean antibody concentration of 22.0 IU/ml.

#### 4.4.4.4 Characteristics of Donors with Persistently High Antibody Levels

There was no clear relationship between the persistence of antibody level above 10 I.U/ml and the interval since the last tetanus boost but 75% of donors with preimmunisation antibody levels above 8 IU/ml appeared to maintain an antibody level above 10 IU/ml after boosting for at least six months (Fig.4.4.2).

Of the 21 men and 12 women who were regularly plasmapheresed, nine men (43%) and three women (25 %)



**Figure 4.4.2**

Distribution of plasma donors with post-immunisation tetanus antibody levels greater than 10 i.u/ml who were still donating in the six months following immunisation

Donors are allocated to groups according to their pre-immunisation tetanus antibody level.

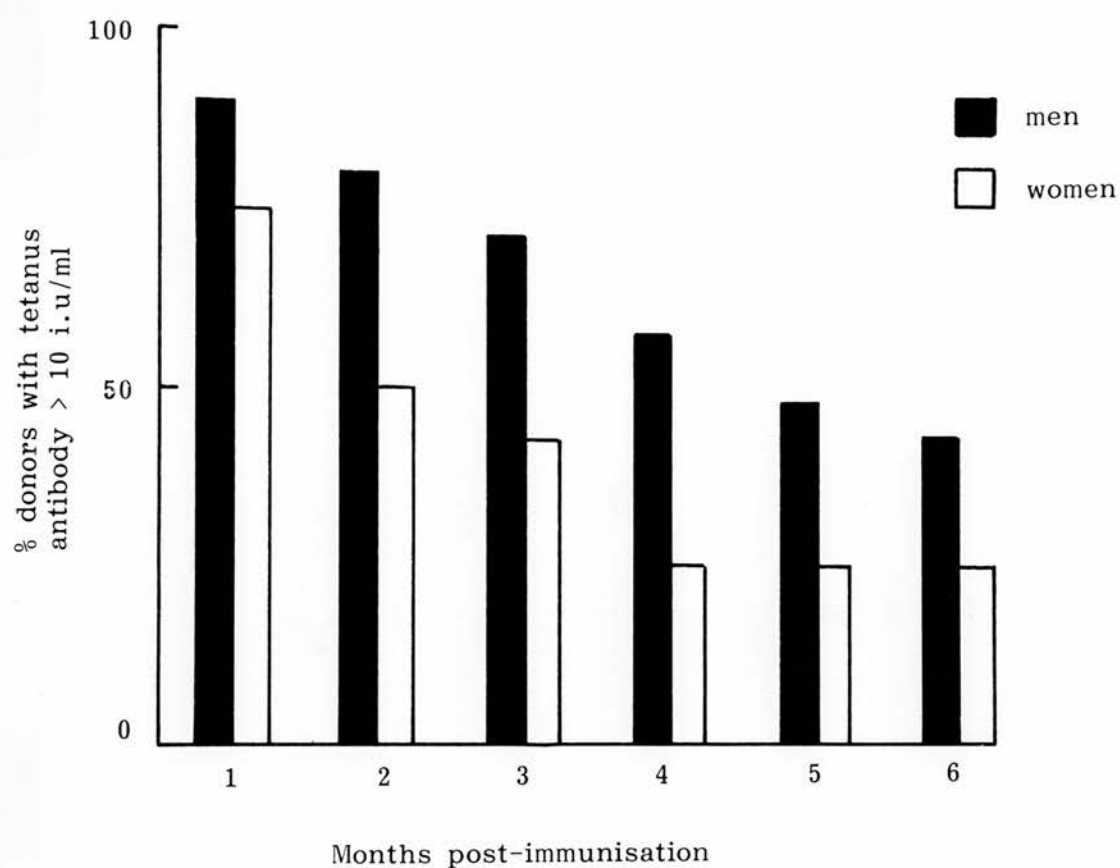
still possessed antibody levels above 10 IU/ml after six months of plasmapheresis (Fig 4.4.3).

#### 4.4.5 Discussion

##### 4.4.5.1 Data From Vaccine Trial

There was no difference in clinical reactivity and immunogenicity between the two vaccines. In this study the overall incidence of local reactions (83.8%) was higher than that found in a smaller study (68%) in which a similar method of recording reactions was used (Deacon et al, 1982 ). Of the 29 non-immune vaccinees who failed to develop antibodies to the toxoid, 18 had no memory of any tetanus vaccination, seven said that they might have been vaccinated during schooldays but not in the last 25 years, and four clearly remembered initial vaccination or 'booster' doses but not within the last 10 years; 24 of the 29 were at least 30 years of age. It may be that many of the non-responders may not have been able to mount a secondary immune response since they would not necessarily have received routine tetanus immunisation in infancy.

The lack of difference between the two vaccines in this study, together with the lower incidence of reactions with plain formol tetanus toxoid (Collier et al, 1979; Rubin et al, 1980) suggest that local reactions may be due to the toxoid itself and/or to inflammatory responses to aluminium hydroxide rather



**Figure 4.4.3**

Sex distribution of donors with post-immunisation tetanus antibody levels greater than 10 i.u/ml during the first six months following immunisation

than to the impurities that are co-purified with the toxoid in the conventional purification technique and which are removed by antibody-affinity purification. This is consistent with previous studies in which the severity and frequency of reactions were not thought to be reduced by further purification of the toxoid (White et al, 1973; Griffith, 1967).

#### 4.4.5.2 Data from Plasmapheresis Donors

The frequency of unselected donors with a tetanus antibody level above 10 IU/ml is very low, ranging from 1.5% (Entwistle and Eldridge, 1973) to 2.8% (Sgouris et al, 1966), so that random screening of suitable donors is an inefficient method of finding immune plasma donors. A programme of hyperimmunisation of plasma donors with tetanus toxoid would overcome the difficulty in recruiting tetanus antibody plasma donors (Cook et al, 1976; Rubin et al, 1980). However, before such a programme is set up, it is important to identify the donors who are unlikely to suffer from severe adverse reactions and who can be expected to produce good antibody levels for a long time after boosting.

The results of this study do not show any statistical difference between the sexes in terms of antibody response to tetanus toxoid. However, the increase in antibody level following boosting with tetanus toxoid was significantly less in those over the



age of 40 years. The reduced antibody response with increasing age has previously been observed (Peel et al, 1978; Yeni et al, 1978).

It is generally difficult to compare reaction rates in different tetanus vaccine studies because of differences in the vaccines and vaccinees, and in the methods of assessing adverse reactions (Collier, 1980). Published reports show that women are more susceptible to reactions than men (Editorial, Br Med J 1974; White et al, 1973), and that there is a higher frequency of reactions in persons with high levels of preimmunisation tetanus antibody (Faktor et al, 1973 ; Levine and Edsall, 1981). This present study confirms that women are more likely to experience adverse reactions than men. In addition, there was a trend towards a higher frequency of adverse reactions with increasing pre-immunisation antibody levels, but this was only significant for moderate and severe pain in the group of vaccinees with a preimmunisation antibody level above 8 IU/ml. This study also suggests unexpectedly that the highest and most significant incidence of reactions with preimmunisation tetanus antibodies is in the group with preimmunisation antibody levels between 0 and 2 IU/ml. This finding has not been previously reported. Since this increased frequency was observed equally in recipients of either immuno-purified or conventionally purified vaccine, it may not be related to vaccine

preparation. These reactions may be an Arthus-type phenomenon (Editorial, Br Med J, 1974), where it is possible that in this antibody range the ratio of antibody to antigen in some subjects was such that immune complexes were formed and caused the reactions.

Although among the donors with persistently high tetanus antibody levels (above 10 IU/ml) six months after boosting there was a preponderance of men and of donors with a preimmunisation antibody level above 8 IU/ml, it is difficult to draw any definite conclusion as the numbers are small. Furthermore, it is not surprising that following boosting, individuals with high preimmunisation antibody levels would maintain a high antibody level longer than those with lower pre-immunisation antibody levels.

This study confirms that boosting of volunteer donors for the production of plasma containing high levels of tetanus antibody produces only minor reactions and that individuals with high tetanus antibody levels can be hyperimmunised without an excess of adverse reactions. Boosting is therefore appropriate in situations where it is difficult to recruit adequate plasma donors for tetanus antibody. Finally, donors most suited for hyperimmunisation with tetanus toxoid are likely to be young males preferably under 25 years of age, with a preimmunisation tetanus antibody level around 8 IU/ml.

#### 4.5 CONCLUSION

Plasmapheresis offers a practical means of meeting the growing demand for plasma for the manufacture of immunoglobulins.

The results from the three studies strongly suggest that boosting of selected blood donors for the procurement of hyperimmune plasma is not only feasible but also a desirable alternative. Indeed, in areas where it is difficult to recruit plasma donors with suitably high antibody levels because the prevalence of these antibody levels in the population is low, hyperimmunisation of selected donors may be the only way to ensure that adequate volumes of plasma can be collected.

At present, donors are withdrawn from the plasmapheresis panel for medical reasons or because their antibody levels have significantly decreased. The latter group of donors could be retained as hyperimmune donors after boosting with vaccine, thus reducing the need for recruiting new donors. Furthermore, because the antibody levels achieved after boosting are so high, fewer donors are needed. Consequently, plasmapheresis programmes are more efficiently run because fewer plasmapheresis procedures are performed and fewer donors need close monitoring.

However, the procurement of any hyperimmune plasma should be individually examined in the light of the

results of the three studies. In certain cases, for example rabies hyperimmune plasma, it is unlikely that random screening of the blood donor population would yield adequate numbers of suitable donors, and hyperimmunisation is the only means whereby hyperimmune plasma could be obtained. On the other hand, there may not be any need for a hyperimmune preparation when the level of antibody in the general population is already high, as in the case of measles immunoglobulin. Table 4.5.1 summarises the present position regarding the suitability of hyperimmunisation of donors for the procurement of hyperimmune plasma.

Active immunisation has long been an accepted practice for the acquisition of immunity against infectious diseases. The dangers of immunisation are well known but have been accepted because of the benefits afforded to individuals and society. Adverse reactions to vaccines are likely to result from sensitisation to the active component of the vaccine, to other proteins that co-purify with the vaccine, or to the adjuvant in the vaccine. They can be so mild as to be unnoticed by the vaccinee or so severe as to manifest as anaphylactic shock.

Moreover, little is known about the effects of hyperimmunisation in man. Immunisation and hyperimmunisation of donors in order to obtain immunoglobulin for passive protection of others offer no

<u>HYPERIMMUNE GLOBULIN</u>	<u>SCREENING</u>	<u>VACCINATION</u>
Tetanus	yes	yes
Hepatitis B	yes	yes
CMV	yes	no
Rabies	no	yes
Varicella-Zoster	yes	yes
Rubella	yes	yes
Measles	yes	yes

Table 4.5.1      Methods of recruiting hyperimmune donors  
for hyperimmune plasma procurement

apparent physiological advantages to the donors and expose them to potential risks. Furthermore, the repeated administration of antigen may theoretically result in vasculitis and amyloidosis (Beebe et al, 1964; Peeler et al, 1965; Bishop et al, 1966; Curran, 1969; Penny and Hughes, 1970). However, to date these complications have not been reported, nor are they likely to occur in plasma donors who have been hyperimmunised.

In view of these potential problems, there is a need for a boosting policy for the procurement of each specific hyperimmune globulin. First of all, the vaccine used for boosting need to have a proven safety record in addition to being immunogenic. Secondly, the policy will define the total number of times that boosting will be undertaken and the minimum time interval between boosting in order to further reduce the likelihood of adverse reactions. This will be based on the findings from boosting studies that have been designed to identify donors most suitable for such programmes.

Nonetheless, it is mandatory that any donor should undergo a full medical examination before entry into any plasmapheresis programme to ensure that his/her health is unlikely to be adversely affected by the plasmapheresis or by hyperimmunisation. Before each plasmapheresis, the donor's serum protein, liver transaminase and haemoglobin levels are measured, and at

two-monthly intervals the physician in charge reviews each donor's physical condition and the accumulated laboratory data to determine whether he/she should continue in the programme. If adverse effects of plasmapheresis are noted, the donor is advised to obtain personal medical care.

In addition to the tests performed on all plasmapheresis donors, serum electrophoresis and quantitative immunoglobulin estimations are performed every four months. Administration of antigen or plasmapheresis is discontinued if these concentrations fall below or rise significantly above the normal range established by the laboratory. Such close monitoring is crucial to safeguard the health of donors.

Whenever a new procedure, including the use of vaccines for boosting donors, is introduced by the SNBTS for the procurement of hyperimmune plasma, the Association of British Insurers are informed. The Association investigates the procedure, and if accepted, will recommend to all its members that there should be no penalty against the donor's life insurance policy in the event of serious adverse reactions. Furthermore, in the unlikely event that a donor suffers serious reactions, there are well-defined procedures to be followed if the donor wishes to claim for financial loss or for injury. Claims are considered by a panel of assessors. After consideration, a recommendation is

reached. If an ex gratia payment should be made, the amount is related to what is currently being awarded for similar injury in the courts. This recommendation is made through the Scottish Home and Health Department to the Treasury.

In conclusion, where a safe and effective vaccine is available it is more efficient to hyperimmunise selected donors for the procurement of hyperimmune plasma, particularly in situations where the prevalence of high specific antibody levels is low in the donor population. However, if a safe and effective vaccine does not exist the procurement of plasma for the manufacture of each specific hyperimmune globulin will depend on random screening of large donor populations however inefficient the latter may be. Finally, the safety of the donor should remain the primary concern in all plasmapheresis programmes.



5    CLINICAL EVALUATION OF AN  
INTRAVENOUS IMMUNOGLOBULIN PREPARATION

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## 5.1 PRIMARY HYPOGAMMAGLOBULINAEMIA

Patients with primary hypogammaglobulinaemia suffer from recurrent infections. They have low levels of serum immunoglobulin at presentation, usually of IgG and IgA isotypes. Table 5.1.1 lists the various types of primary hypogammaglobulinaemia. Only the two more common types will be discussed below in detail ; X-linked hypogammaglobulinaemia, and late onset primary hypogammaglobulinaemia.

### 5.1.1 X-linked hypogammaglobulinaemia

In X-linked hypogammaglobulinaemia, also known as Bruton agammaglobulinaemia (Bruton, 1953), or Swiss type agammaglobulinaemia, the presentation is during the first two years of life and usually after the first three months. It is a recessive disease of variable clinical severity, characterised by low serum IgG and virtually absent IgA and IgM, absent B-cells in the peripheral blood (Froland and Natvig, 1972; Preud'homme et al, 1973), absent plasma cells in the lymphoid tissues (Wedgwood, 1978), and normal cell immunity (Gotoff, 1968). Patients have low C1q levels because of a shortened half-life of the C1q secondary to the low immunoglobulin levels (Kohler and Muller-Eberhard, 1972).

Boys with X-linked hypogammaglobulinaemia suffer from recurrent sino-pulmonary infections, as well as

---

Predominantly antibody defects

- 1 X-linked hypogammaglobulinaemia
- 2 X-linked hypogammaglobulinaemia with growth hormone deficiency
- 3 Autosomal recessive agammaglobulinaemia
- 4 Immunoglobulin deficiency with increased IgM (and IgG)
- 5 IgA deficiency
- 6 Selective deficiency of other immunoglobulin isotypes
- 7 K-chain deficiency
- 8 Antibody deficiency with normal gammaglobulin levels or hypergammaglobulinaemia
- 9 Immunodeficiency with thymoma
- 10 Transient hypogammaglobulinaemia of infancy
- 11 Common variable immunodeficiency

Humoral deficiency associated with other defects

- 1 Transcobalamin II deficiency
- 2 Ataxia Telangiectasia
- 3 Wiskott-Aldrich Syndrome

---

Table 5.1.1 List of predominantly primary humoral immunodeficiency diseases

more serious infections such as septicaemia, meningitis and osteomyelitis. They recover normally from childhood exanthematas, but they are unduly susceptible to enteroviruses, for example chronic echovirus infection and vaccine-associated poliomyelitis. They may present with chronic diarrhoea or sprue-like syndromes and associated protein-losing enteropathy as a result of persistent gastrointestinal infections with rota-virus (Saulsbury et al, 1977) or Giardia lamblia (Ochs et al, 1972).

#### 5.1.2 Late onset primary hypogammaglobulinaemia

Late-onset primary hypogammaglobulinaemia (also known as CVI) refers to hypogammaglobulinaemia affecting the three main classes starting after two years of age, but it can occur at any age and has an equal incidence in males and females. It is often familial but no clear mode of inheritance has been established. There is a high incidence of lupus, haemolytic anaemia, and idiopathic thrombocytopenic purpura among first degree relatives. Patients with this condition suffer from the same sino-pulmonary infections as patients with X-linked hypogammaglobulinaemia, but they also suffer from other complications such as polyarthrititis, autoimmune diseases for example pernicious anaemia, and more severe gastrointestinal disturbances including nodular lymphoid hyperplasia and

malignancy. Patients with this condition also have an increased incidence of sarcoidosis or of a sarcoid-like syndrome (Leen et al, 1985b).

B-lymphocytes are present in about 20% of patients and are below 6% in a further 25% (Preud'homme et al, 1973; Horwitz et al, 1977). Up to a third of these patients have a T-lymphocyte defect as shown by poor lymphocyte transformation to mitogen and by negative delayed hypersensitivity skin tests (Webster and Ascherson, 1974). However, despite these abnormalities these patients are not usually more susceptible to infection than those with normal in vitro T-cell function.

#### 5.1.3 Frequency of primary hypogammaglobulinaemia

The MRC estimated that the prevalence of primary hypogammaglobulinaemia in the United Kingdom was around 1 per 100,000 (MRC, 1971). The prevalence for Sweden is thought to be at least 2 per 100,000 (Bjorkander et al, 1984). Figures for Japan, Denmark, and Italy are even lower ( Hayakawa et al, 1981; Koch et al, 1981; Luzi et al, 1983).

#### 5.1.4 Hypogammaglobulinaemia and replacement therapy

All patients with hypogammaglobulinaemia benefit from prophylactic immunoglobulin replacement therapy (MRC 1971). In children, the treatment limits irreversible lung damage (Bjorkander et al, 1984), and



prevents serious infections such as septic arthritis, osteomyelitis and meningitis. In adults immunoglobulin replacement is beneficial although it does not always prevent exacerbation of chest infections in patients with chronic lung damage. However, if these patients do not receive immunoglobulin replacement, they may die from fulminant pneumonia (Ascherson and Webster, 1980). The benefit is dose-dependent (MRC, 1971; Nolte et al, 1979; Pirofsky et al, 1980; Cunningham-Rundles et al, 1984). Patients with low levels of IgG subclasses with or without concomittant IgA deficiency who suffer from respiratory infections have also benefitted from immunoglobulin prophylaxis (Schur et al, 1970 ; Oxelius, 1974; Hanson et al, 1980). Finally, Buckley (1983) found that a group of patients with normal levels of IgG and IgG subclasses who do not develop specific antibodies on immunisation may also be helped by immunoglobulin prophylaxis.

The optimal dose and dosage frequency of IgG prophylaxis is still unknown. This is probably due to the variable degree of mucosal and lung damage in these patients, the variability of the clinical expression of the disease, and other underlying disorders that may accompany the syndrome. This is also reflected by the variable half-life of infused IgG reported in these patients (Schiff et al, 1984).

### 5.1.5 Immunoglobulin replacement

Immunoglobulin may be administered either intramuscularly, subcutaneously, or intravenously. This can also be effected in the form of plasma infusions.

#### 5.1.5.1 Intramuscular Route

The MRC have conducted a trial of IM IgG replacement therapy, comparing the effects of high (50 mg/kg/week), medium (25 mg/kg/week), and low (12.5 mg/kg/week) replacement in a large number of patients with primary hypogammaglobulinaemia (MRC, 1971). The trial of the low dose was stopped because it was ineffective. Patients receiving the high dose had significantly fewer febrile episodes, respiratory and skin infections and diarrhoea; their weight was higher and C-reactive protein levels (a non-specific indicator of inflammation) was lower. The current British practice is based on this trial. After a loading dose of 50 mg/kg daily for five days, 25 mg/kg is given every week by IM injection. If recurrent infections continue the dose is increased to 50 mg/kg weekly or twice weekly. For a 60 kg individual, the volume of IgG for intramuscular injection is 20 ml, and is often accompanied by severe pain. Patient compliance was therefore poor.

#### 5.1.5.2 Subcutaneous Route

In view of the problems associated with

immunoglobulin replacement via the IM route, other methods of replacement were attempted. Immunoglobulin can be slowly infused subcutaneously by means of a constant infusion pump. This may achieve good serum IgG levels and can be used in patients with poor venous access. More importantly, the patients can administer the treatment themselves just like the diabetics use an insulin infusion pump (Berger et al, 1980). However, not all patients are capable of using the infusion pump satisfactorily, and some batches of immunoglobulin for IM use are associated with pain and mild inflammation at the site of administration, possibly due to kinin release (McClelland, personal communication).

#### 5.1.5.3 Plasma Infusion

Immunoglobulin can be administered in the form of plasma, but carries the risk of transmitting viral diseases, such as HBV and NANB hepatitis (Kirkpatrick, 1980 ; Holland et al, 1981), and of sensitisation in the recipients to proteins in the plasma. Wells and Buckley (1977) reported an anaphylactic reaction in a young girl with hypogammaglobulinaemia within minutes of receiving 15 ml of ABO-compatible paternal plasma. Subsequent analysis of the patient's pre-treatment serum revealed that it contained an anti-IgA antibody titre of 1 in 640. Some of the risks of viral disease transmission may be reduced but not eliminated by using a "buddy" system

where a small panel of up to four healthy carefully selected blood donors are plasmapheresed at regular intervals to provide sufficient plasma that is equivalent to 90 mg/kg of immunoglobulin every three to four weeks. The advantages of this method include delivery of substantial amounts of immunoglobulin of all classes, including IgA and IgM, but fluid overload may be a limiting factor in patients with cardiovascular and renal diseases, and limiting the number of donors would limit the antibody repertoire administered to the patient.

#### 5.1.5.4 Intravenous Immunoglobulin

This form of replacement therapy has been advocated as the treatment of choice for three main reasons; firstly, serum IgG levels are immediately raised; secondly, very high serum levels of IgG can be reached as there is no volume limitation ; and finally, there is minimal discomfort for the patient. The main drawback until recently has been the availability of immunoglobulin preparations that are safe for IV use. Clinical trials seem to confirm the efficacy of IV preparations over IM ones. Most of this difference in efficacy is probably due to the higher dose of immunoglobulin which can be administered, and to the fact that some of the immunoglobulin, which administered by the IM route, may be subjected to local

proteolysis.

Finally, it will be quite possible that in future patients will be able to self-administer the IV IgG in the comfort of their own homes, just as haemophiliacs administer their own Factor VIII.

#### 5.1.6 Characteristics of immunoglobulin

There are five classes of human immunoglobulin: IgG, IgA, IgM, IgD, and IgE. Immunoglobulins in clinical use are mostly made up of IgG with traces of IgA and IgE.

##### 5.1.6.1 Structure and Function of Immunoglobulin

Immunoglobulin molecules are made up of equal numbers of heavy and light polypeptide chains held together by non-covalent forces and usually by covalent interchain disulphide bridges to form a bilaterally symmetrical structure (Figure 5.1.1). The class and subclass of an immunoglobulin molecule is determined by its heavy chain type.

Each polypeptide is made up of a number of loops or domains of rather constant size (100-110 amino acid residues) formed by intrachain disulphide bonds. The N-terminal of each chain is called the variable region as there is a wide variation in amino acid sequence in that region.

Papain cleaves the IgG molecule in the hinge region between the C $\gamma$ 1 and C $\gamma$ 2 domains to give two identical

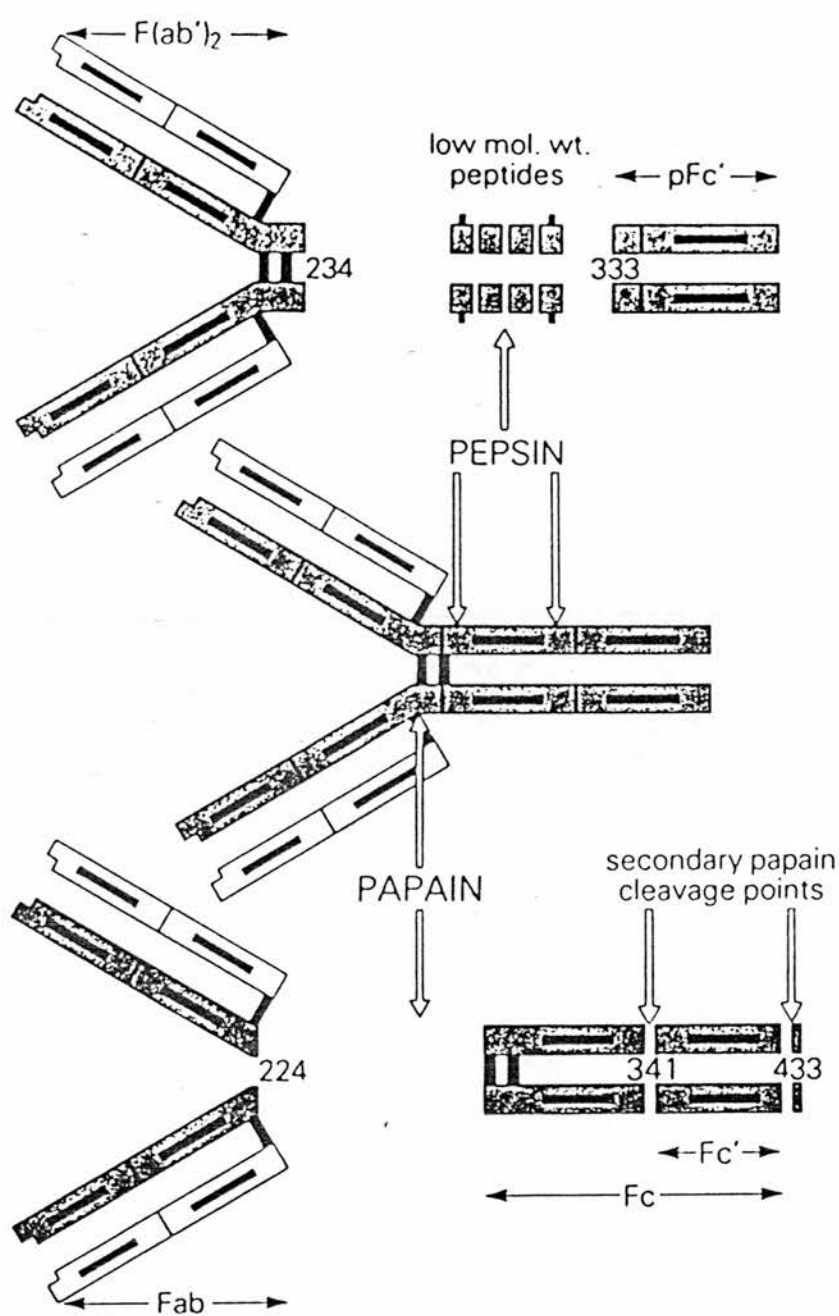


Figure 5.1.1

Digestion of IgG molecule by pepsin and papain

Fab fragments which include an entire light chain and the VH and CH1 domains of a heavy chain; one Fc fragment, composed of the C terminal halves of the heavy chains (Porter, 1959)

Pepsin splits the molecule on the C-terminal side of the inter-heavy chain disulphide bonds into a large F(ab)2 fragment made up of two Fab fragments (Nisonoff et al, 1960). The F(ab)2 fragment broadly encompasses the two Fab regions linked by the hinge region, and the pFc' fragment corresponds to the C $\gamma$ 3 domain of the molecule. The Fc fragment is extensively degraded by pepsin (Figure 5.1.1).

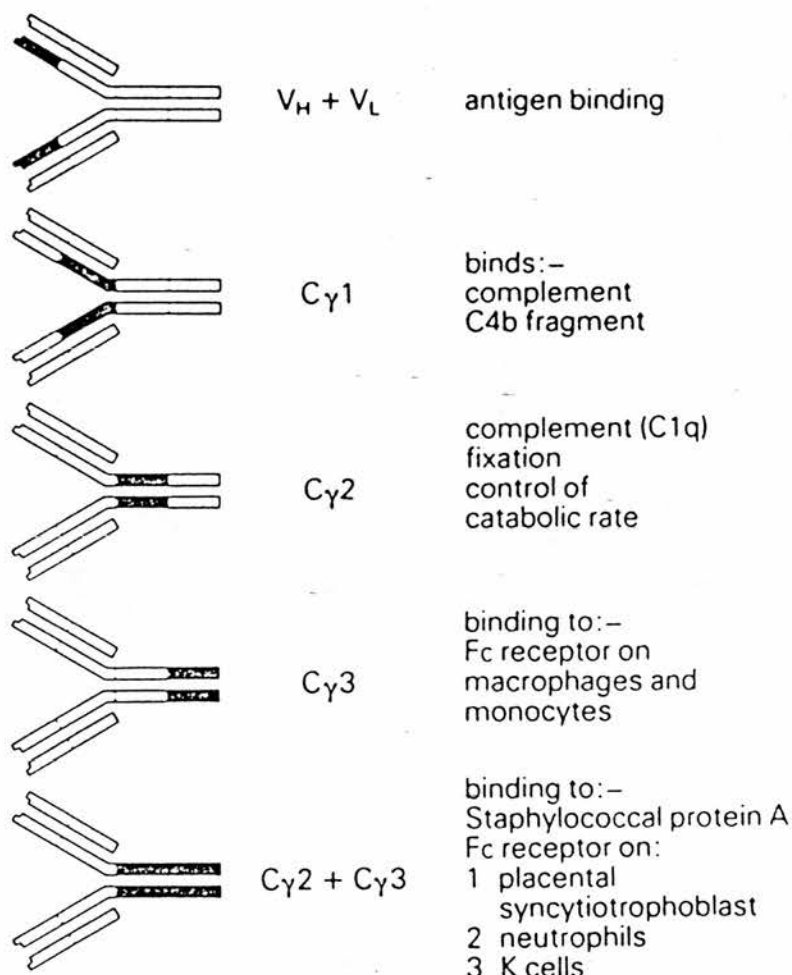
Antigen binding activity is associated with the Fab part of the molecule and most of the secondary biological activities of the molecule such as complement fixation, monocyte binding and placental transmission are mediated through the Fc part.

Each domain acts as a functional subunit which subserves a specific function. VH and VL domains interact to form the antigen binding surfaces of the antibody molecule. The functions performed by the other domains are summarised in Figure 5.1.2.

IgG makes up 20% of total plasma protein and 70-75% of total immunoglobulin pool in man. It is a monomeric protein with a sedimentation coefficient of 7s and a molecular weight of 146,000. It is evenly distributed between the intra- and extravascular pools, and is the

domains

function



**Figure 5.1.2**

**Functions performed by the various domains  
of the IgG molecule**



major antibody of the secondary immune responses. It is also the only immunoglobulin that can cross the placenta in humans, and it is responsible for the protection of the newborn during the first few months of life.

There are four subclasses of IgG with different structure, properties and biological functions. They are found in different proportions in normal serum with the relative concentrations of IgG1, 60-70% ; IgG2, 14-20% ; IgG3, 4-8% ; and IgG4, 2-6% (Steinberg et al, 1973). The subclasses have different numbers and arrangements of the interchain disulphide bonds.

IgG can fix serum complement with the different subclasses differing in their ability to do so. IgG4 is completely unable to fix complement by the classical pathway but may be active in the alternative pathway. It is also able to harness basophils and mast cells. IgG2 is thought to be the predominant subclass in response to bacteriae with polysaccharide capsules. IgG3, although present in small amounts, is a powerful activator of complement.

#### 5.1.7 Manufacture of intravenous immunoglobulin

Although there is controversy over what constitutes a safe IV immunoglobulin preparation, it is generally agreed that the design of a new intravenous immunoglobulin preparation should meet the following basic requirements:

- 1 All the general pharmacopoeial requirements for human normal IgG
- 2 Low IgG aggregates
- 3 Low contamination with components of contact activation system
- 4 Preservation of functionally intact IgG molecules
- 5 Freedom from microbial agents.

#### 5.1.7.1 General Pharmacopoeial Requirements

The requirements for human immunoglobulin were laid down in 1966 by the WHO expert committee on Biological Standardisation (WHO Technical Report Series, 1967).

Immunoglobulins are prepared from plasma of at least 1000 healthy donors from intact placentae or from retroplacental blood. The large number of donors is required to ensure that the antibody profile is representative of the population. The fractionation method should yield a product which is free from the transmission of infection and should concentrate the antibodies in the starting pool by at least ten times. The final product should also be free from any contaminant likely to be present in any plasma derivative, for example, bacterial toxins (pyrogens), and/or prekallikrein activator (PKA). Physico-chemical requirements include at least 90% intact IgG molecule with normal biological activities, and a well balanced

distribution of IgG subclasses.

The requirements mean that new immunoglobulin preparations are developed from Cohn Fraction II (Cohn et al, 1946), as this method of immunoglobulin preparation is the only one which has consistently been shown to yield a product with a low risk of hepatitis transmission (Finlayson, 1979)

#### 5.1.7.2 Low Immunoglobulin Aggregates

Standard IgG produced by Cohn fractionation consists mainly of IgG monomers but variable amounts of polymers and aggregates are also produced during fractionation. Severe anaphylactoid reactions observed after IV infusion of standard IgG preparation, are thought to be due to large molecular weight IgG aggregates that spontaneously activate the complement system resulting in the release of vasoactive compounds (Barandun et al, 1962). Therefore, low levels of IgG aggregates are desirable to reduce the likelihood of anaphylactoid reactions.

#### 5.1.7.3 Low Contamination with Components of the Contact Activation System

Alving et al (1980) noticed that vasoactive reactions following IV infusions of standard IgG were similar to those observed following the infusion of plasma protein fraction (Alving et al, 1978). These reactions were therefore attributed to the presence of a

component of the kallikrein-kinin system, PKA. When rapidly infused, PKA acts on circulating prekallikrein which in turn cleaves bradykinin from high molecular weight kininogen. This reaction is self-generating because of several feedback mechanisms and results in large amounts of bradykinin causing reactions in the recipient, for example pain at injection site, flushing, and dyspnoea.

However, adverse reactions to IV immunoglobulin cannot always be attributed to PKA levels or to the aggregate content of the individual IgG preparation. Eibl (1985) showed that IV immunoglobulin preparations containing very high levels of PKA (5 to 10 times the PKA content of the implicated batches of plasma protein fraction reported by Alving) were not necessarily associated with adverse reactions. Nonetheless, in view of the fact that these enzymes can cause reactions and because of the availability of tests to detect them, it is generally agreed that IV IgG preparations should contain low levels of vasoactive enzymes.

#### 5.1.7.4 Functionally Intact Molecules

It is important that during the manufacture of an IV immunoglobulin preparation the resultant IgG molecule should be as unmodified as possible so that it will maintain all the essential functions of the native IgG molecule. In this respect, the manufacture of

IV IgG using enzymatic degradation may lead to a preparation devoid of Fc receptor effector activity, and other manufacturing processes may yield preparations deficient in certain IgG subclasses (Liehl et al, 1981; Romer et al, 1982a).

#### 5.1.7.5 Methods of Producing Intravenous Immunoglobulin Preparations

Because immunoglobulins prepared by standard fractionation methods are not well tolerated when infused intravenously (Barandun et al, 1962), gammaglobulin fractions obtained by these methods have to be further processed to achieve a product suitable for IV use.

Several new immunoglobulin preparations have been developed for IV use. Four main procedures are employed to ensure safe IV use :

##### 1 Physical methods

Selective elimination of IgG aggregates can be achieved by precipitation with polyethylene glycol (PEG) or PEG with hydroxyethyl starch (HES), by adsorption on DEAE-Sephadex with subsequent stabilisation by albumin,

Preparations treated with PEG or PEG/HES tend to have some residual anticomplementary activity, due to molecular reaggregation during storage. Furthermore, PKA and kallikrein levels are not reduced by this process.

Purification of Cohn Fraction II by ion exchange chromatography on DEAE-Sephadex removes IgA as well as aggregates. Reaggregation is prevented by the addition of human albumin as stabiliser. Although some aggregates are still detectable, the product is free of anticomplementary activity. In addition, this product has the added advantage of being the only one known to be devoid of IgA. However, IgG4 subclass concentration is relatively low. It is believed that this subclass, which focusses in a narrow acidic pH region, is eliminated by the DEAE-Sephadex treatment in the final purification (Romer et al, 1982a).

## 2 Chemical modification of the IgG molecule

IgG molecules can be chemically modified by beta-propiolactone (Stephan, 1975) or by cleavage of the interchain disulphide bridges by sulphonation (Masuho et al, 1977), or by reduction and alkylation (Fernandes and Lundblad, 1980).

Modification of the IgG molecule by beta-propiolactone causes alkylation of histidyl and acylation of lysyl residues. The molecule retains the Fc region and some of its effector function, but IgG3 subclass, which is more susceptible than other IgG subclasses to denaturation or degradation by plasmin, sulphonation, or treatment with beta-propiolactone, is lost (Skvaril et al, 1980).

An alternative method of chemical modification is selective cleavage of the interchain disulphide bonds with sulphite and tetrathionate ions. The IgG molecules are believed to retain the molecular structure of the intact IgG through non-covalent interaction and to have normal *in vivo* half-life (Kobayashi et al, 1981). Cutter Laboratories have developed a method whereby interchain disulphide bonds are split (Schroeder et al, 1980). Mild reduction cleaves three to five interchain bonds in the hinge region leaving intrachain disulphide linkages unaffected; alkylation of the free -SH groups with iodoacetamide prevents reformation of the disulphide bonds, and results in a stable product which has low anticomplementary activity and low PKA.

However, beta-propiolactone is classified as a carcinogen, and iodoacetamide is toxic. It is therefore essential to ensure that the final product is entirely free of such toxic reagents.

### 3 Enzymatic degradation

The IgG molecule undergoes enzymatic degradation by plasmin (Sgouris, 1967) or pepsin (Schultze and Schwick, 1962) in order to remove the Fc part of IgG.

IV IgG preparation manufactured by enzymatic degradation results in a product devoid of anti-complementary activity. Although such preparations can irreversibly bind antigen, the immune complexes they

form can activate complement and in vitro studies have shown that these preparations may not necessarily protect against infection (Painter et al, 1966). In addition, they have a shortened in vivo half-life, thereby limiting their clinical usefulness (Barandun, Skvaril and Morell, 1975).

Plasmin cleaves the IgG molecule into two identical monovalent Fab fragments and one Fc fragment (Figure 5.1.1). The therapeutic effects depend essentially on the 30-40% of the IgG molecules which are resistant because of the differing degree of plasmin resistance in the IgG subclasses (Merler et al, 1967; Barandun, Skvaril and Morell, 1975).

Pepsin cleaves the IgG molecule at a site immediately adjacent to the disulphide linkages joining the two heavy chains, leaving a product consisting of approximately 80% divalent F(ab)<sub>2</sub> and 20% Fc fragments. These fragments can form antigen-antibody complexes but cannot activate complement. They are well tolerated when infused intravenously but they have a shortened half-life of 18 hours (Janeway et al, 1968). At neutral pH, pepsin is inactive and therefore there is no further cleavage of IgG molecules during storage in solution.

Enzymatically digested IgG preparations are highly fragmented, with a short half-life. They have a limited clinical usefulness, but are probably ideal for the neutralisation of toxins, or for the removal of toxic



drugs, for example in the treatment of digoxin poisoning (Smith et al, 1982) .

#### 4 Enzyme treatment at pH 4.0

Incubation at pH 4.0 leads to mild denaturation which removes anticomplementary activity. The exact mode of action is not entirely understood, but it is thought that the acid medium activates inherent plasma proteases contaminants. IgG preparations manufactured by incubation at pH 4.0 for 48 hours have been shown to contain low anticomplementary activity but under certain conditions may be contaminated with high levels of PKA and IgG aggregates. However, these aggregates are not anticomplementary in vitro or in vivo, and in mouse protection studies no noticeable difference is observed between pH 4.0 and standard preparations (Barandun et al, 1975).

This method has been modified by the addition of trace amounts of porcine pepsin during pH 4.0 incubation. The presence of the enzyme causes aggregate dissociation but little or no fragmentation (Painter and Law, 1984). A protein stabiliser (carbohydrate) is added during titration and subsequent freeze-drying to prevent reaggregation or denaturation of the IgG molecules.

##### 5.1.7.6 Manufacture of SNBTS IV IgG

The IV IgG produced by the Protein Fractionation

Centre of the SNBTS is manufactured by further processing of Cohn Fraction II.

In brief, 0.9% sodium chloride is added to Cohn Fraction II to reduce the protein concentration to 50 g/l. The resultant solution is then sterilised by filtration through a series of filters.

## 1 Ultrafiltration

This sterile solution is then subjected to ultrafiltration to remove residual ethanol. The ultrafiltration is performed in an Amicon DC 10 hollow fibre dialyser/concentrator using a H10X50 cartridge with nominal molecular weight cut-off of 50,000. The resultant solution contains less than 10 mg of ethanol per gram of protein, a concentration which is known to cause little or no denaturation on extended storage. The IgG concentration at this stage is within the range 90 to 120 g/l.

Maltose is added immediately to the above protein solution at a concentration of 2 g maltose per gram of dissolved protein. This step is necessary to stabilise the protein solution with respect to spontaneous aggregate generation during further processing and in particular during the pH 4.0 incubation step.

## 2 Acid hydrolysis and pepsin treatment

The solution is then titrated to pH 4.0 with 0.5 M hydrochloric acid. Freshly prepared porcine pepsin

solution is then added to the solution before the whole mixture is sterilised by membrane filtration.

The resulting sterile solution is incubated at 35 C for at least 20 hours, after which it is slowly retitrated to pH 6.9 with 0.5 M sodium hydroxide. The solution is then sterilised through a 0.2 $\mu$  membrane filter before freeze-drying. The final product after reconstitution is a solution of 5% (5g/100ml) human normal immunoglobulin with less than 5% aggregates and at least 95% monomer and dimer. The mean anticomplementary activity is 28 mg/CH50.

#### 5.1.8 Overall Study Design

After an initial trial in five healthy volunteers, and following approval from the Physicians Ethics Committee, a study was undertaken to evaluate the safety and clinical efficacy of SNBTS IV IgG in patients with primary hypogammaglobulinaemia.

In view of the large number of different types of primary humoral immunodeficiency (Table 5.1.1), some of which are very rare, this study was limited to patients belonging to the two more common types of primary hypogammaglobulinaemia ; X-linked hypogammaglobulinaemia and CVI.

The initial assessment of the efficacy of this IV IgG preparation having been previously carried out (Yap et al, 1985), the main objectives of the study were:

- 1 To assess the tolerance and incidence of adverse reactions to the IV administration of SNBTS IV IgG
- 2 To detect hepatitis events by monitoring the serum ALT levels in patients following treatment with SNBTS IV IgG
- 3 To increase the serum IgG level into the normal range in patients with hypogammaglobulinaemia with SNBTS IV IgG therapy
- 4 To assess the clinical response to SNBTS IV IgG.

With an estimated incidence of primary hypogammaglobulinaemia of the order of 1 to 2 patients per 100,000 population, and probably with many of the mildly affected patients undiagnosed, there was only a limited number of patients available for study throughout Scotland. The study took place between February 1983 and November 1985. As physicians and other transfusion centres became aware of the existence and availability of SNBTS IV IgG, and learned of its potential benefit, more patients were gradually enrolled into the study. Therefore, the number of patients in the study increased from nine in early 1983 to 25 by mid-1985.

Following referral to the South East Scotland Regional Blood Transfusion Centre, patients underwent a full medical examination and were assessed regarding the need for IV IgG therapy.

Twenty-one of the 25 patients were assessed by myself and I reviewed the case notes and radiographs of the other four patients. A clinical review of all the patients was important to confirm the diagnosis in each case, and to examine any interesting clinical features that might indicate an effect due to the IV IgG therapy, for example, frequency of infection.

The first infusion of IV IgG was usually carried out in the Regional Centre, under close medical supervision. The second and subsequent infusions were carried out in the patient's local hospital. Pre- and post-infusion blood samples and nursing observations performed during the infusions were sent to the Regional Centre by first class post or special delivery. Detailed progress reports were obtained from the patients' physicians and I was very closely involved in the clinical management of the severely affected patients, and visited those who were critically ill.

A profile of the patients entered in the study is outlined in Appendix A. Patients who participated in the studies described in sections 5.2, 5.3, 5.4 and 5.5 are shown in Table 5.1.2.

Two patients (Cases 8 and 23) moved away from Scotland three and nine months respectively after they were entered in the study. Another patient (Case 25) died in a car accident 23 months after he was entered in the study.

Patient No.	Sex	Diagnosis	Case Number in Section			
			5.2	5.3	5.4	5.5
1	F	CVI*	1			
2	M	CVI	2	1	1	1
3	M	CVI	3			
4	F	CVI	4	2	2	2
5	F	CVI	5	3		3
6	M	CVI	6		3	4
7	F	CVI	7	4		5
8	M	CVI	8		4	
9	M	CVI	9	5		6
10	F	CVI	10			
11	M	CVI	11	6	5	7
12	M	CVI	12			
13	M	CVI	13	7		8
14	M	XL**	14	8		9
15	M	XL	15	9		10
16	M	XL	16	10		11
17	F	CVI	17	11		12
18	M	CVI	18			
19	F	CVI	19	12	6	13
20	F	CVI	20	13	7	14
21	M	CVI	21	14		
22	M	XL	22	15		15
23	F	CVI	23			
24	M	XL	24	16		16
25	M	CVI	25	17		17

\* CVI- common variable immunodeficiency

\*\* XL - X-linked hypogammaglobulinaemia

Table 5.1.2 List of patients with the corresponding case numbers in the studies described in sections 5.2, 5.3 and 5.5. Further details are given in Appendix A.

## 5.2 STUDY ON ACUTE TOXICITY (ADVERSE REACTIONS)

### 5.2.1 Introduction

Early in the clinical use of immunoglobulins, clinicians became aware that the IV administration of immunoglobulin was associated with a high incidence of adverse reactions (Janeway, 1970). Furthermore, adverse reactions to immunoglobulin occurred more frequently in patients with primary hypogammaglobulinaemia than in subjects with normal serum immunoglobulin levels (Barandun et al 1962).

### 5.2.2 Causes of Reactions

The underlying mechanism of adverse reactions to infused immunoglobulin has been a source of controversy. Barandun et al (1980) described adverse reactions in newly diagnosed patients after their initial infusions of IV immunoglobulin manufactured by mild pepsin treatment at pH 4.0 (Sandoglobulin). On the basis of the aetiology, Barandun et al (1980b) have attempted to differentiate four main types of adverse reaction : anaphylactoid, hypersensitive, vasoactive and phlogistic.

#### 5.2.2.1 Complement Activation

Anaphylactoid reactions occur almost exclusively in patients with hypogammaglobulinaemia and are thought to be triggered by IgG aggregates that have anti-

complementary activity. IgG preparations containing aggregates which spontaneously activate complement in vitro cause anaphylactoid reactions in hypogammaglobulinaemic patients when administered intravenously under standardised conditions (Barandun et al, 1962). These reactions include flushing of the face, dyspnoea, lower back pain, nausea, vomiting followed by fever and in some cases circulatory collapse and loss of consciousness. These symptoms, which are usually observed immediately after the start of the infusion, are accompanied by a moderate but discernable decrease in the total haemolytic complement activity.

#### 5.2.2.2 Hypersensitivity

Hypersensitive reactions are due to antibodies of the recipient, particularly those directed against IgA (Buckley, 1980) or other immunogenic substances in the IgG preparation (e.g. thiomersal). Hypersensitive reactions to thiomersal are now non-existent as IgG preparations no longer contain thiomersal.

#### 5.2.2.3 Vasoactive Contamination

Some complications are caused by vasoactive or microbial contaminants (pyrogens, endotoxins, bacteria, etc) of immunoglobulin preparations that have undergone inappropriate production and/or handling procedures. PKA and kallikrein contaminating IV IgG preparations have been suggested as causes for vasoactive reactions



(Alving et al, 1980a). Eibl (1985), however, provided data suggesting that the administration of IV IgG contaminated with a very high level of PKA was not necessarily followed by adverse reactions.

#### 5.2.2.4 Phlogistic Reactions

Finally, certain reactions result from inflammatory phenomena representing physiological antigen-antibody reactions in the recipients (so called phlogistic reactions). Barandun et al (1980a) also observed a marked exacerbation of both local and systemic inflammatory symptoms following the IV administration of immunoglobulin in newly diagnosed hypogammaglobulinaemic patients suffering from clinically evident infections. Such patients are believed to be in a state of relative antigen overload, and the rapid IV infusion of a large dose of antibodies capable of reacting with their corresponding antigens in the blood and tissues may thus intensify the inflammatory processes as a consequence of the formation of antigen-antibody complexes. Typical phlogistic reactions are characterised by fever 1 to 3 hours after the onset of the infusion, transient increase in pre-existing local inflammations (e.g. arthritis, pneumonic infiltrations, erythema, etc) and rise in white cell count. Subjective symptoms disappear after a few hours without therapy and objective signs after one to two

days.

Various different immunoglobulin preparations have since been developed for safe IV administration. SNBTS IV IgG, an intravenous immunoglobulin prepared by mild pepsin proteolysis at pH 4.0 and freeze-dried in the presence of maltose as a stabiliser, is one such preparation (see section 5.1.7.3). The tolerance and incidence of adverse reactions to SNBTS IV IgG were assessed in patients with primary hypogammaglobulinaemia.

### 5.2.3 Patients and Methods

Twenty-five patients (16 male, 9 female; age range 11-64 years) were assessed in this study from February 1983 to November 1985, with each patient receiving SNBTS IV IgG therapy for periods ranging from five to 34 months (mean 19.8 months). In brief, 20 suffered from late onset primary hypogammaglobulinaemia (CVI) and five from X-linked hypogammaglobulinaemia (table 5.1.2). Consent was obtained from the local hospital Ethics Committee, the patient's own physician, and from the patient prior to the commencement of the study.

#### 5.2.3.1 Infusion Dose and Rate

In all but eight patients a total dose of 0.2 g/kg body weight SNBTS IV IgG was administered on each occasion. Two of the eight patients received up to 0.4 g/kg of IV IgG at later infusions as their serum IgG was

below 3 g/l when receiving an infusion dose of 0.4 g/kg. One further patient received an even higher dose of 0.54 g/kg during subsequent infusions because of his persistently low serum IgG on a dose of 0.4 g/kg. Four patients were given 0.1 to 0.15 g/kg IV IgG at their first infusion because they had not previously received any immunoglobulin replacement of any kind. They received 0.2 g/kg body weight at their second and subsequent infusions. One further patient received 0.05 g/kg IV IgG at his first infusion because he had not previously received any immunoglobulin replacement, and in addition had a history of allergic reactions to insulin and numerous antibiotics. He received 0.1 g/kg of IV IgG at his second infusion and 0.2 g/kg IV IgG at subsequent infusions.

Infusions were commenced at a rate of 15 mg/kg/hr with a doubling of the rate 15, 30 and 60 minutes after the start of the infusion. During their first infusions, five patients who had previously never received any form of immunoglobulin therapy were given IV IgG at an infusion rate of 7.5 mg/kg/hr, and the rate was doubled every 15 to 30 minutes to 120 mg/kg/hr. Second and third infusions were generally commenced at a rate of 30 mg/kg/hr and 60 mg/kg/hr respectively, with the rate gradually increased to 120 mg/kg/hr, as they could tolerate this without adverse reactions. In two patients, the infusion rate was increased to 240

mg/kg/hr and 264 mg/kg/hr to assess the incidence of adverse reactions at the increased rates.

Infusions were initially administered at three-weekly intervals and subsequently varied according to the patient's clinical state to maintain a serum trough (pre-infusion) immunoglobulin level at around 3 g/l. The frequency of infusion therefore ranged from one week to five weeks.

#### 5.2.3.2 Patient Assessment

The oral temperature, pulse and blood pressure were monitored during the initial infusion at 0, 15, 30, 45, and 60 minutes and half-hourly thereafter until the end of the infusion. Patients were also asked to record any abnormal sensations felt in association with the IV Ig infusions.

#### 5.2.3.3 Immunological Assays

Assays of total haemolytic complement (CH50), C3, and C4 were carried out on most patients immediately before and after their first infusions, and in those patients who received the infusions at a higher rate (greater than 120 mg/kg/hr). C3 and C4 were measured by laser nephelometry (Whicher et al, 1978) and haemolytic complement (CH50) was measured by an automated method (Fischer, 1967).

#### 5.2.4 Results

Of a total of 738 infusions, 704 were free of any significant reactions. Patients received five to 77 infusions (mean 29.5) each over the study period. Adverse reactions were associated with 34 of the SNBTS IV IgG infusions (4.6%), and were mostly mild and encountered in 10 out of 25 (40%) patients. There were 30 episodes of mild fever associated with the infusions, and four patients (Cases 2, 5, 6 and 20) felt shivery after their first infusion (Table 5.2.1). The mild reactions were usually observed in the initial IgG infusions for nine of the 10 patients with 21 of the 34 adverse reactions (62%) occurring within the first five infusions in each of the patients (Table 5.2.1).

Four adverse reactions, experienced by three patients, were moderately severe. The reactions were not related to any particular production batch of IV IgG, and no adverse reactions were reported by other patients receiving similar batches of IV IgG. One patient (Case 6) had never been treated with any form of immunoglobulin replacement therapy prior to SNBTS IV IgG and felt unwell a few hours after his first two infusions, experiencing influenza-like symptoms, with shivering, sweating and generalised muscle aches. However, his third and fourth SNBTS IV IgG infusions were immediately followed by prophylactic oral aspirin (1200 mg) and this abolished all adverse reactions. In

	Infusions	Patients
Total Number	738	25
Adverse reactions	34 (4.6%)	10
moderately severe	4 (0.5%)	3
mild fever	30 (4%)	9
shivery symptoms	7 (0.9%)	4

Table 5.2.1 Adverse reactions after SNBTS IV IgG infusions

the fifth infusion no aspirin prophylaxis was administered, and there was no adverse reaction.

Another patient (Case 20) who had never previously had any form of immunoglobulin replacement felt nauseated, and dizzy during her first SNBTS IV IgG infusion. She felt better once the infusion rate was reduced and remained well after the infusion rate was later increased according to the infusion protocol. However, she vomited after her infusion. Although she was pyrexial four hours after the completion of the infusion, she did not experience any other adverse reactions and remained asymptomatic. All her subsequent infusions were uneventful although on one occasion she felt dizzy when the infusion rate was in excess of the rate recommended in the protocol, and her symptoms disappeared when the rate of infusion was reduced to 120 mg/kg/hr.

One further patient (Case 15) developed an urticarial macular rash one hour after the commencement of his 39th infusion. The infusion was stopped immediately and he received 10 mg of chlorpheniramine maleate and 10 mg of metoclopramide because of the itchy rash and nausea. His temperature and blood pressure were normal. A tachycardia of 120 per minute was noted but became normal 45 minutes after the infusion was stopped. He did not have a pyrexia, and complement studies before and after the infusion showed no significant changes.

Although the rate of infusion was 240 mg/kg/hr (twice the usual rate), he had received similar infusion rates on at least six previous occasions without any reactions and without any changes in serum complement levels. His 40th and 41st infusions, 10 days and 21 days later, were uneventful although the infusion rate was increased slowly from 120 mg/kg/hr to a maximum of 240 mg/kg/hr by increments of 60 mg/kg/hr every 30 mins. All subsequent infusions at a rate of 240 mg/kg/hr were similarly without adverse reactions.

Complement studies did not show any changes to suggest complement activation during any of the infusions (Figures 5.2.1, 5.2.2 and 5.2.3) including those where adverse reactions had been recorded (see Table 5.2.2).

#### 5.2.5 Discussion

Immunoglobulin replacement therapy for patients with primary hypogammaglobulinaemia has up to now been mainly by the intramuscular route. However, due to the limited volume of immunoglobulin that can be administered by this route, (usually 25 - 50 mg/kg/week) a proportion of such patients still suffer from recurrent infections.

In previous studies, a 59% incidence of adverse reactions observed in recipients of an IV IgG preparation containing large quantities of polymeric IgG



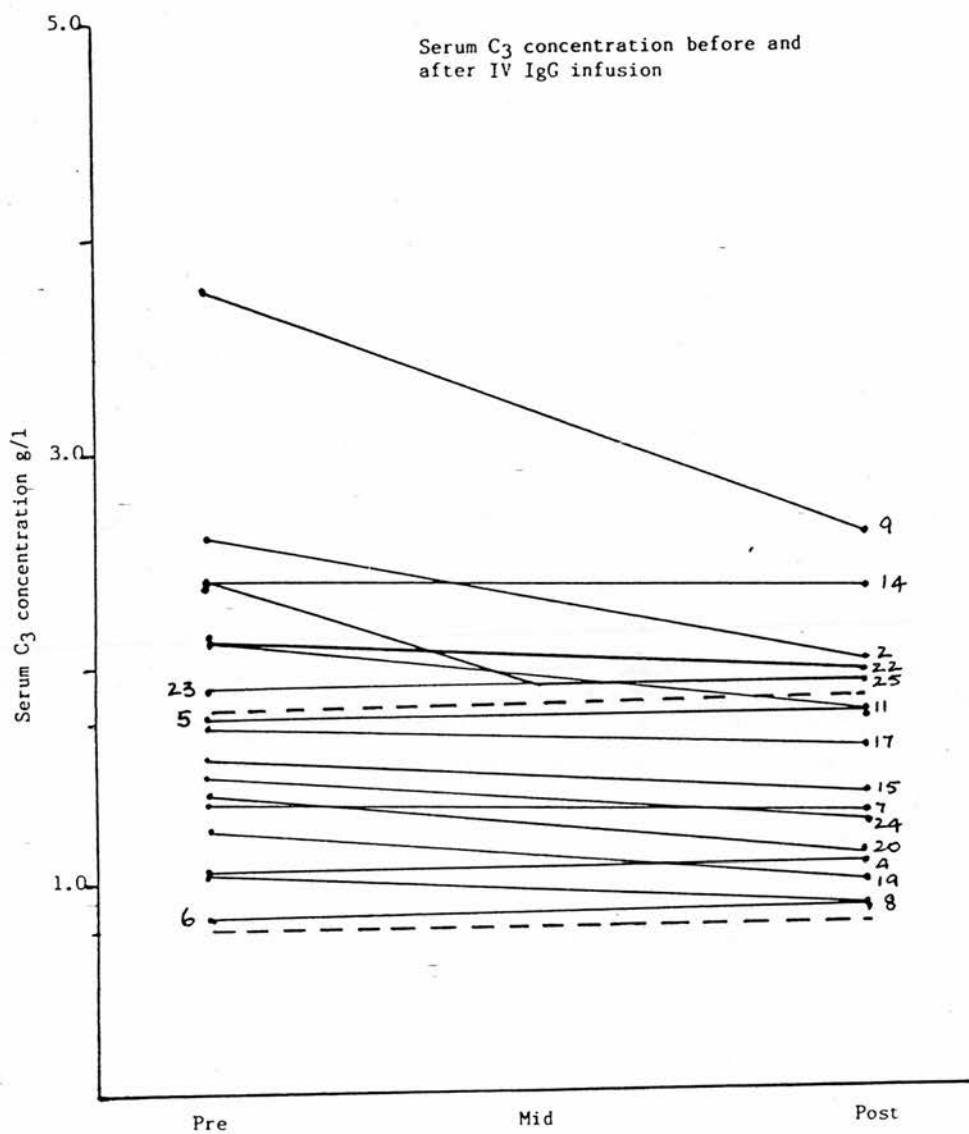


Figure 5.2.1

Pre- and post-infusion serum C<sub>3</sub> values

Serum  $C_4$  concentrations before and after IV IgG infusion

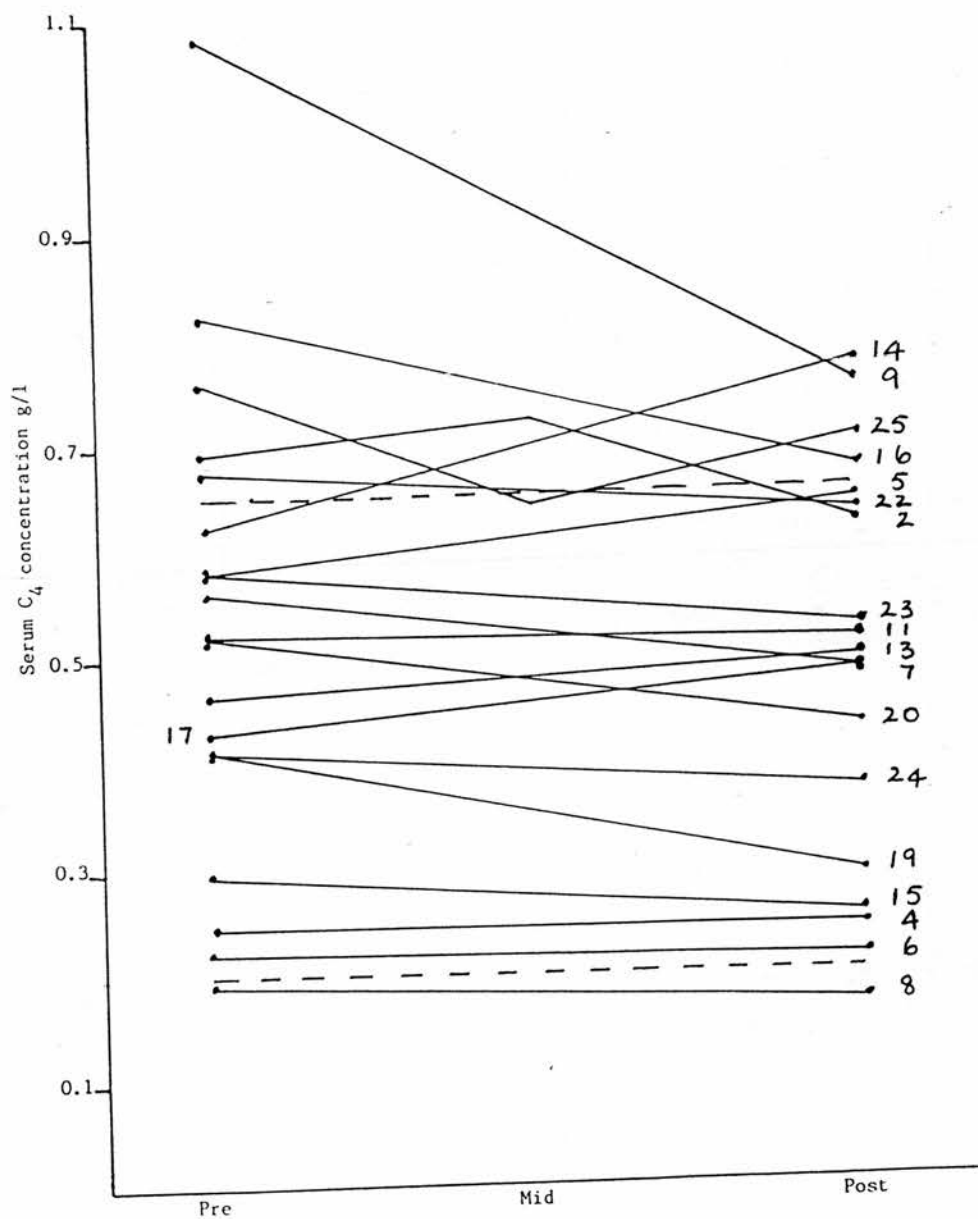


Figure 5.2.2

Pre- and post-infusion serum  $C_4$  values

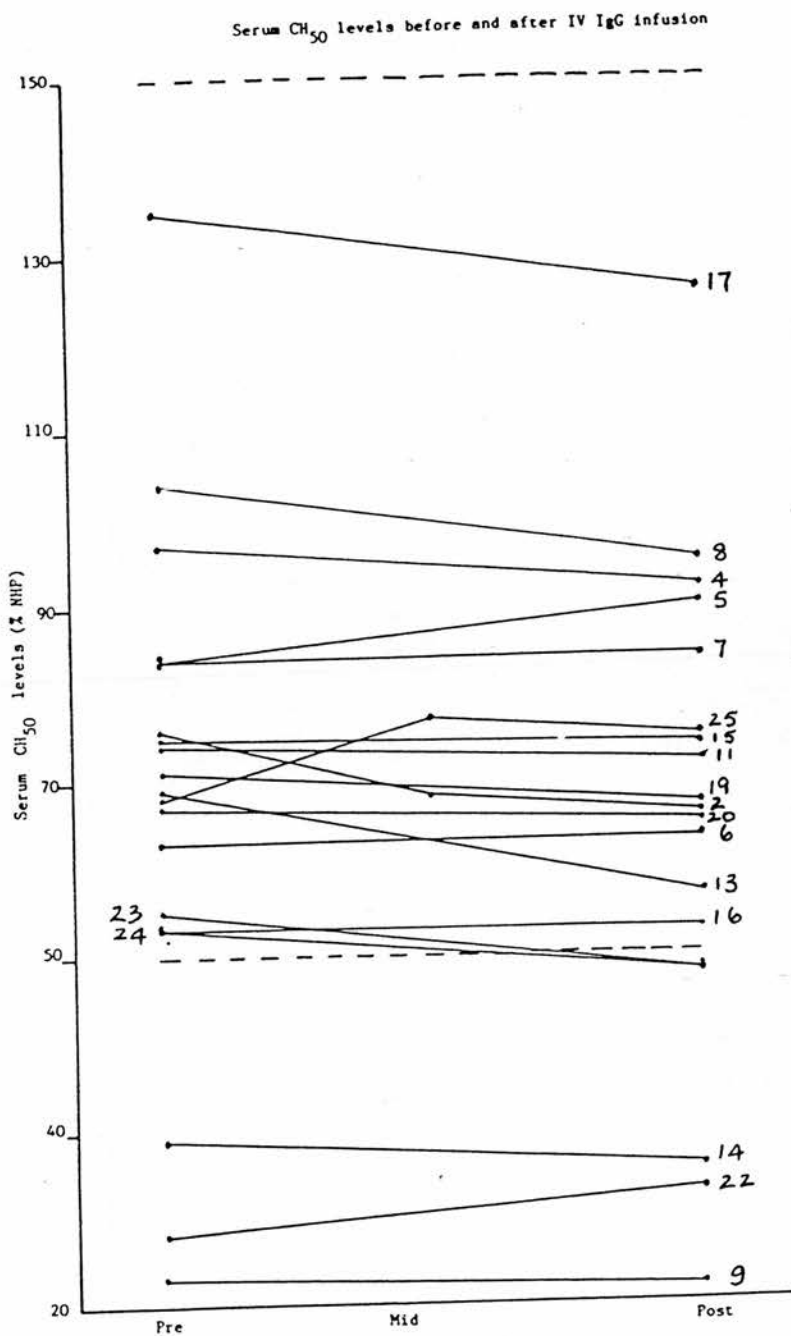


Figure 5.2.3

Pre- and post-infusion serum  
haemolytic complement values

Case No.	CH50 (50-150)		C4 (0.20-0.65 g/l)		C3 (0.76-1.60 g/l)	
	Pre	Post	Pre	Post	Pre	Post
6*	1300	1300	0.22	0.21	0.84	0.86
15	52	48	0.42	0.37	1.20	1.07
20	67	65	0.52	0.43	1.41	1.11

Values for normal range are shown within parentheses

\* Tests performed by Dr G Bird, Newcastle General Hospital. Normal ranges are:

CH50 - 1260-2880; C4 - 0.14-0.42 g/l; C3 - 0.75-1.5 g/l

Table 5.2.2 Complement levels during infusions associated with adverse reactions

(Ochs et al, 1982) was reduced to 19% when the same manufacturers (Cutter Laboratories) produced a preparation containing lower amounts of polymers and fragments (Ochs et al, 1984). Studies by Eibl et al (1984) and by Amman et al (1982) confirmed that preparations containing mostly IgG monomers and a carbohydrate as stabiliser were associated with fewer adverse reactions. Furthermore, the study by Matsumoto et al (1981) showed the importance of identifying patients who are at increased risk of developing side effects; patients with severe combined immunodeficiency experienced more adverse reactions than patients with X-linked or common variable hypogammaglobulinaemia. These studies suggest that the presence of polymeric IgG, the use of different stabilisers and the population of patients studied are important factors that may affect the incidence of adverse reactions to IV IgG preparations.

Adverse reactions were associated with 34 infusions (4.6%) in patients with primary hypogammaglobulinaemia. Three patients suffered four moderately severe adverse reactions. The other adverse reactions were mostly mild, asymptomatic and consisted of a mild pyrexia. The low incidence of reactions following SNBTS IV IgG infusion is comparable to other intravenous immunoglobulin preparations and is consistent with the low polymer content of preparations manufactured by the method using

mild pepsin treatment at pH 4.0 and with the use of a carbohydrate stabiliser (Barandun et al, 1980a; Cunningham-Rundles et al, 1981). Interestingly as the incidence of adverse reactions in individual patients decreases with increasing number of infusions, and as adverse reactions occur with the initial infusions, studies with a large number of infusions per patient result in a reduced overall incidence of adverse reactions. It would therefore be preferable, when analysing such results, to examine the adverse reactions in terms of the first 10 to 20 infusions.

Barandun et al (1980a) described similar reactions in newly diagnosed patients after their initial infusions of IV IgG manufactured by mild pepsin treatment at pH 4.0 (Sandoglobulin), and postulated that these were due to a reaction of antibody with antigen in tissues which only occurred in patients with clinically evident infections. However, the two patients who experienced the severe adverse reactions at their initial infusions and the patient who developed the itchy rash and nausea during his 39th infusion all appeared free of infection at the time. The probable abolition of reaction in one of the patients by a small prophylactic oral dose of aspirin is consistent with the release of prostaglandin as a mechanism for adverse reactions as proposed by Passwell, Rosen and Merler (1980). The occurrence of nausea and of an itchy rash

in one of the patients in this study during his 39th infusion cannot be fully explained except on the basis of a high rate of infusion, although the patient tolerated the same infusion rate previously and on subsequent occasions.

Although adverse reactions are more frequent during the early infusions in patients with primary hypogammaglobulinaemia, they may still occur at any time during therapy. Concurrent infection, complement activation and PKA were not associated with the adverse reactions in this study.

### 5.3 STUDY ON THE RISK OF HEPATITIS TRANSMISSION

#### 5.3.1 Introduction

Immunoglobulin preparations generally have a good record for safety with respect to the transmission of viral hepatitis (McClelland, 1984). However, there have been reports of NANB hepatitis in patients with primary hypogammaglobulinaemia receiving IV IgG preparations that have been treated so as to make them safe for intravenous use (Lever et al, 1984; Ochs et al, 1985), raising the possibility that NANB hepatitis viruses may not always be inactivated or removed during the manufacture of these immunoglobulin preparations.

In the absence of an acceptable serologic marker for NANB hepatitis, the incidence of hepatitis following the administration of SNBTS IV IgG was examined by regular clinical review of patients with primary hypogammaglobulinaemia and by serial measurements of their serum ALT levels. Furthermore, the patients underwent regular tests for the presence of HBsAg. This was carried out in a prospective study in patients receiving SNBTS IV IgG. In addition, because these patients had received other forms of immunoglobulin replacement including FFP, their serum ALT levels were also evaluated retrospectively.



### 5.3.2 Patients, Materials and Methods

#### 5.3.2.1 Patients

All 17 patients (Table 5.1.2) had proven primary hypogammaglobulinaemia. Details on the patients are outlined in Appendix A. In brief, 16 of the patients who were receiving IV IgG were enrolled in the prospective trial. One patient (Case 14) had received only FFP as replacement therapy.

#### 5.3.2.2 Fresh Frozen Plasma

Plasma was collected for each patient by plasmapheresis of two or four dedicated donors to minimise the risks of transmitting infection including NANB hepatitis. A dose of 15 ml/kg body weight at two- to four-weekly intervals was used. Plasma donors met the standard medical and serological requirements for blood donors, including normal serum ALT levels. The duration of FFP replacement therapy in the eight patients in the retrospective study ranged from 8 months (Case 8) to 13 years (Case 14).

#### 5.3.2.3 SNBTS IV IgG

Details of the intravenous immunoglobulin preparation used (SNBTS IV IgG) are shown in section 5.1.7. Patients received 200 to 400 mg/kg of SNBTS IV IgG every two to five weeks. The duration of SNBTS IV IgG treatment ranged from six to 25 months.

#### 5.3.2.4 Other Immunoglobulin Preparations

Seven of the patients in the study received other IV IgG preparations (Gamimune, Cutter Laboratories; Sandoglobulin, Sandoz) in an earlier evaluation of immunoglobulin therapy (Yap et al, 1985), and 13 of the patients received standard IM IgG manufactured by Cohn fractionation at the SNBTS Protein Fractionation Centre, prior to the study period (Figure 5.3.1).

#### 5.3.2.5 ALT Assays

The majority of the ALT levels were measured either by an enzymatic (kinetic) method on a Multistat III centrifugal analyser using a Boehringer ALT (OPT) kit or by sequential multiple analysis with Computer System (SMAC 2), Technicon Instrument Association. In a small number of instances, ALT levels were measured at the patient's local hospital clinical chemistry laboratories using standard automated methods. The normal range of serum ALT levels is 11-50 U/l.

#### 5.3.2.6 Hepatitis B serology

Venous blood samples were tested for HBsAg by radioimmunoassay prior to the first SNBTS IV IgG infusion and in certain cases again at six to 12 monthly intervals. Blood samples were also tested for antibodies to HBsAg and to core and e antigens.

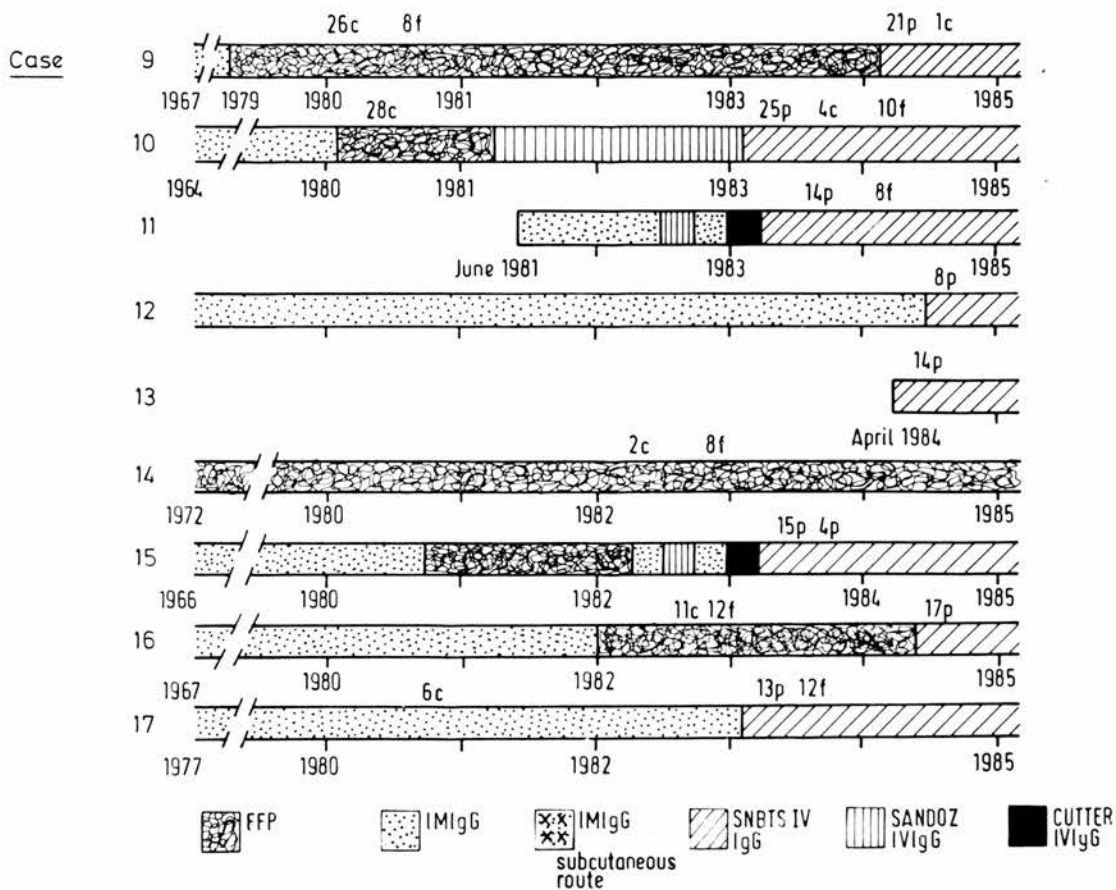
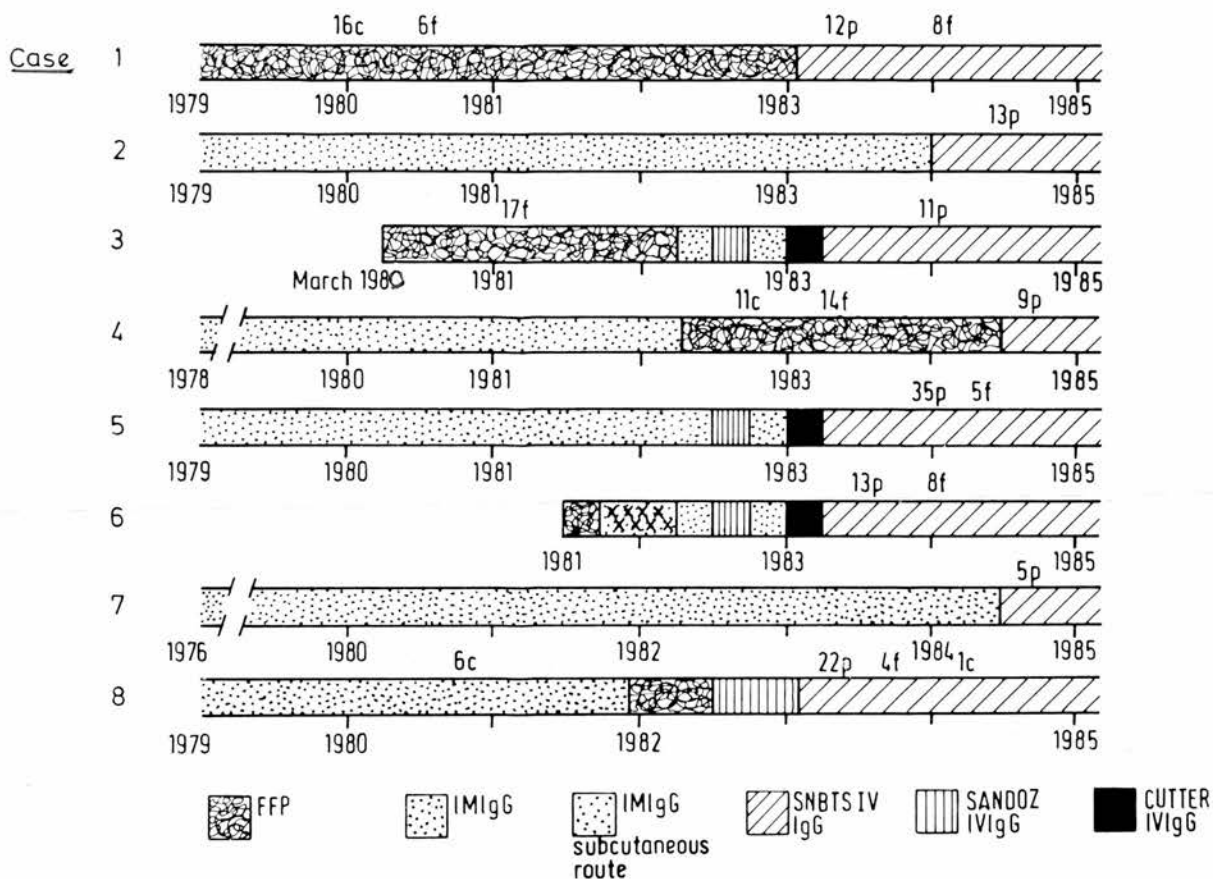
Figure 5.3.1

Types of immunoglobulin in replacement therapy  
received by Cases 1-17

The numbers about the matched lines indicate the number  
of samples in which ALT levels were determined.

c = ALT values obtained for patient case notes  
f = ALT values obtained on samples stored at -150 C  
p = ALT values obtained in a prospective study

Sandoglobulin = Sandoz IVIgG  
Gamimune = Cutter IVIgG



### 5.3.3 Study design

Serum ALT values in the patients who had received FFP therapy were obtained over periods of six to 29 consecutive months in eight patients. The case records were examined first and where no ALT results were recorded, pre-infusion serum samples, stored at -150 C in the vapour phase of liquid nitrogen, were retrieved for ALT assays.

All 16 patients receiving SNBTS IV IgG had serum samples taken before and after each infusion of immunoglobulin for estimation of IgG and ALT levels. The following serum samples were available for analysis;

- 1 From December 1983, ALT levels in all serum samples were measured prospectively
- 2 Before December 1983, ALT levels were obtained retrospectively from patient case records
- 3 Where there were no records of the serum ALT having been previously determined, pre-infusion serum samples (stored frozen at -150 C) were retrieved and ALT levels measured.

Prospective data on ALT levels were therefore available in 11 patients for 13 to 15 consecutive months and in a further five patients for five to 11 consecutive months.

Blood samples from seven patients (Cases 1, 3, 6, 8, 9, 10, 11, and 14) were collected in Edinburgh and the

sera were separated within 24 hours after collection. Blood samples from 9 patients (Cases 2, 4, 5, 7, 12, 13, 15, 16 and 17) were collected outside Edinburgh as these patients received their immunoglobulin infusion at their local hospital and the blood samples were sent by post; the sera were separated immediately on arrival (usually within 24 -72 hours of collection) and kept at 4 C until assayed.

A patient was diagnosed as having hepatitis if the ALT level was above the normal range (50 U/l) in two or more sequential blood samples taken within a 3 to 17 day interval and if one of these levels was at least twice the upper limit of normal (100 U/l). An episode of hepatitis was considered to be probably of viral aetiology if there was no other identifiable cause for the raised ALT (Stevens et al, 1984).

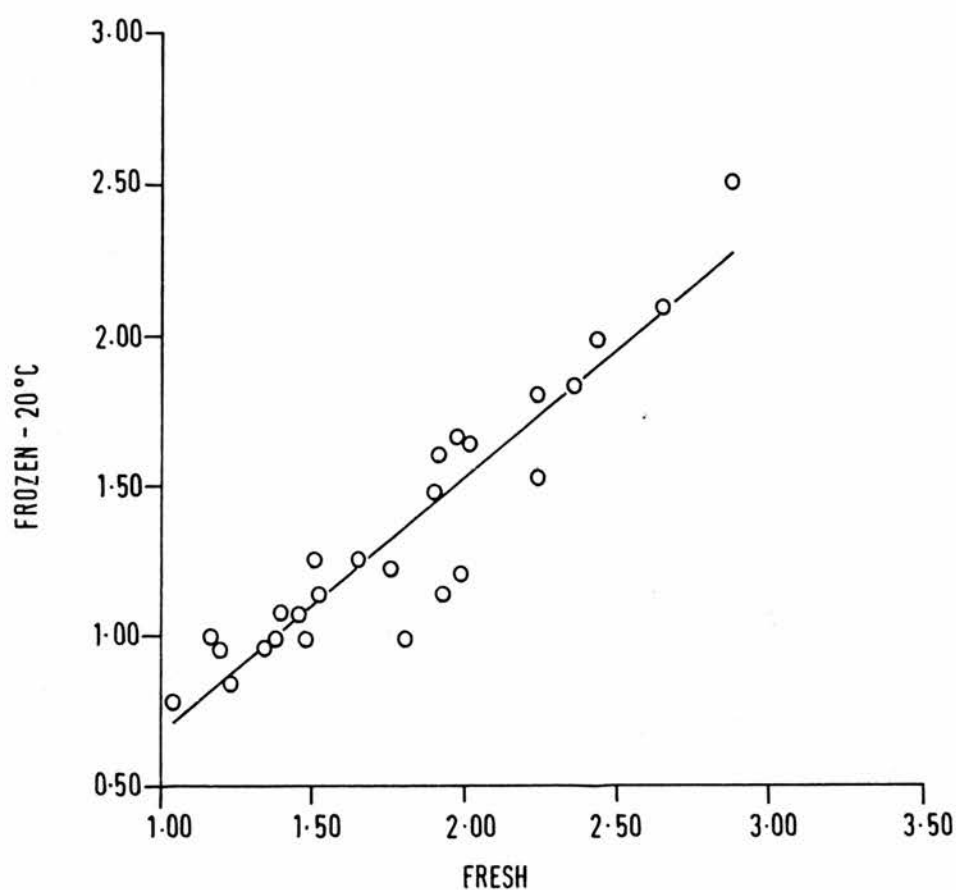
#### 5.3.4 Validity of stored samples

Thirty-one serum samples were collected from patients with liver disease of non-infective aetiologies and sent for ALT assay. Aliquots of the sera were stored at -20 C and at -150 C for at least six months before they were assayed for ALT activity.

#### 5.3.5 Results

##### 5.3.5.1 Validity of Stored Sera

Twenty-six samples stored at -20 C and 28 samples stored at -150 C were available for ALT



**Figure 5.3.2**

**Effect of storage at -20 C for six months  
on serum ALT values**

Horizontal axis : log of serum ALT value when assayed  
fresh

Vertical axis : log of serum ALT value after six  
months storage at -20 C

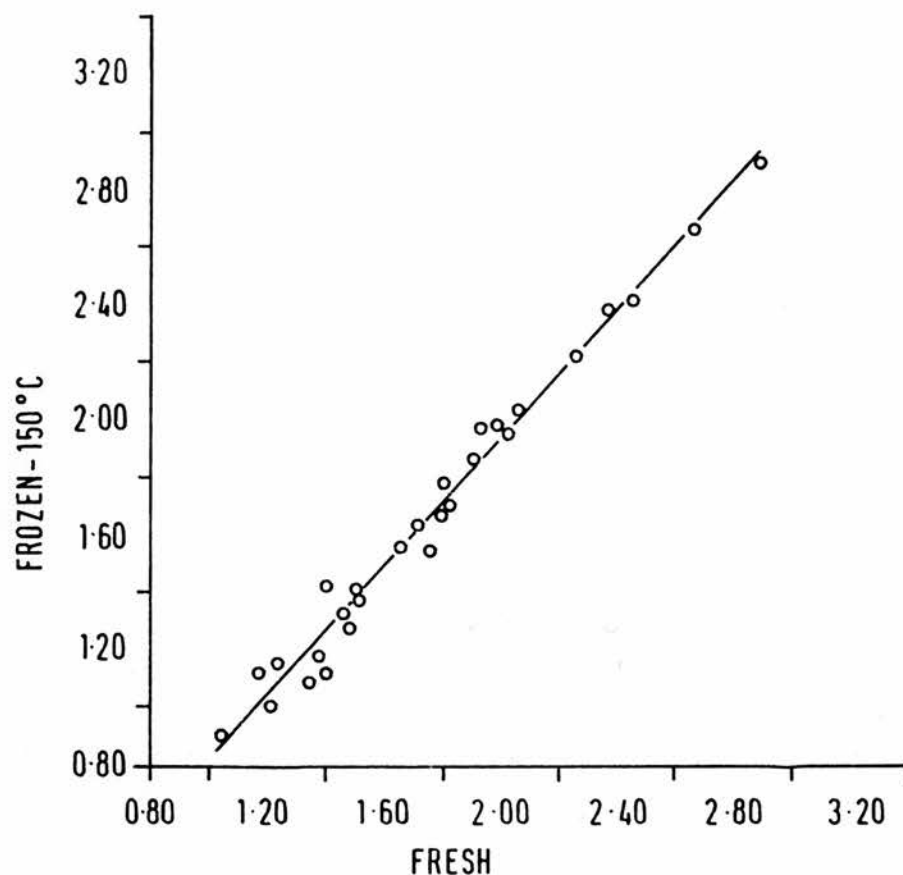


Figure 5.3.3

Effect of storage at -150 C for six months  
on serum ALT values

Horizontal axis : log of serum ALT value when assayed  
fresh  
Vertical axis : log of serum ALT value after six  
months storage at -150 C



measurement. ALT values of samples stored for at least six months at -20 C and at -150 C were compared with the ALT values measured at the time of sample collection (Figures 5.3.2 and 5.3.3). It was found that serum ALT activity decreased after storage at -150 C and at -20 C for up to six months ( $p < 0.001$  by Wilcoxon's rank sum test). The decline in ALT activity was minimal for samples stored at -150 C (a constant decrease of 8 U/l), in contrast to samples stored at -20 C when significant decreases in serum ALT activity were noted (at least 60% of the original value).

#### 5.3.5.2 ALT Levels During FFP Therapy

Among the eight patients who had received FFP therapy in the study, 100 serum ALT levels were available from the case notes: 25 samples (from three patients) had levels above 50 U/l ; two from Case 1 (67 and 55 U/l), seven from Case 9 (range 53-113 U/l) and 16 from Case 10 (range 52-203 U/l). From an additional 65 frozen serum samples sent for ALT measurement only three were abnormal, all from Case 1 (114, 60 and 69 U/l). Therefore, of 165 serum ALT values available for analysis, 28 (from three patients) were abnormal (Figures 5.3.4.a, 5.3.4e and 5.3.4f).

#### 5.3.5.3 ALT Levels During SNBTS IV IgG Therapy

A total of 249 serum samples were obtained and assayed for ALT levels immediately prior to each

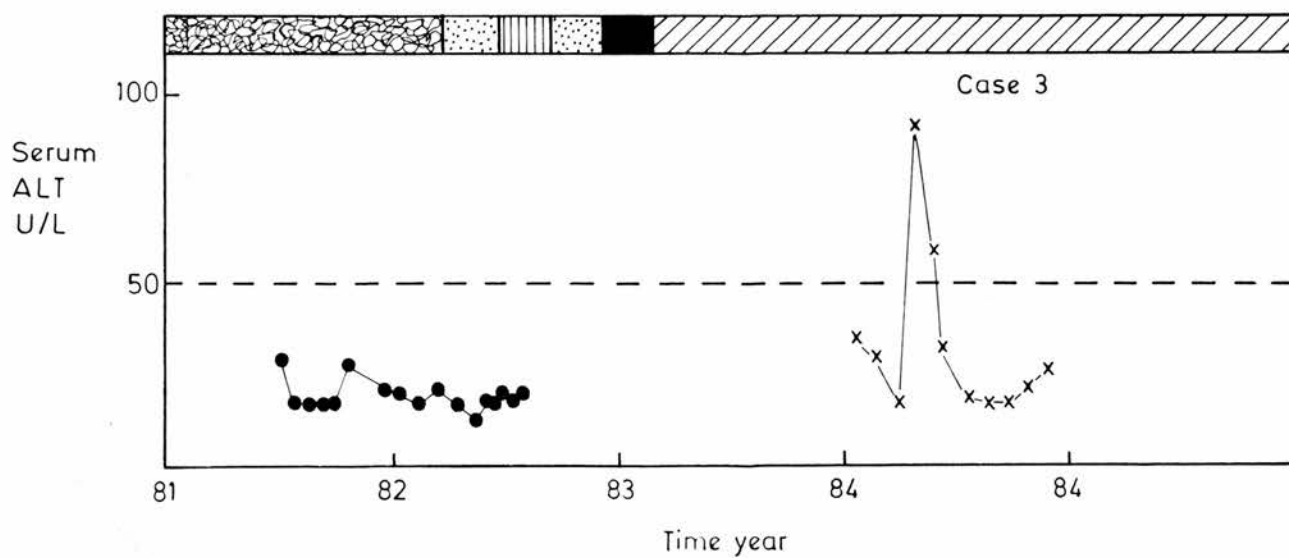
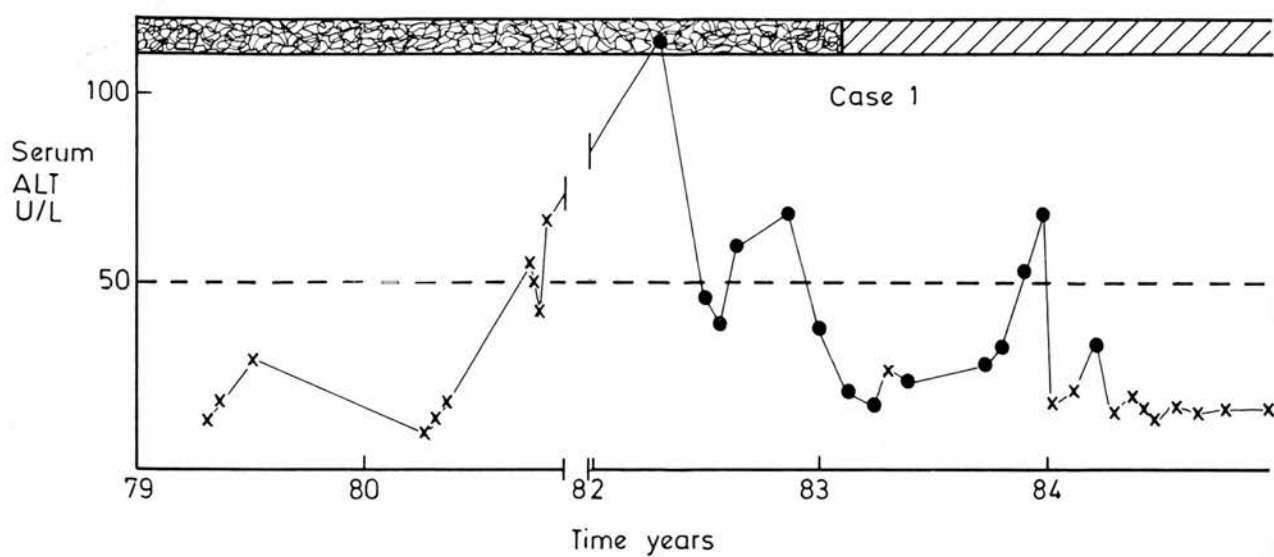
Figure 5.3.4 a - g

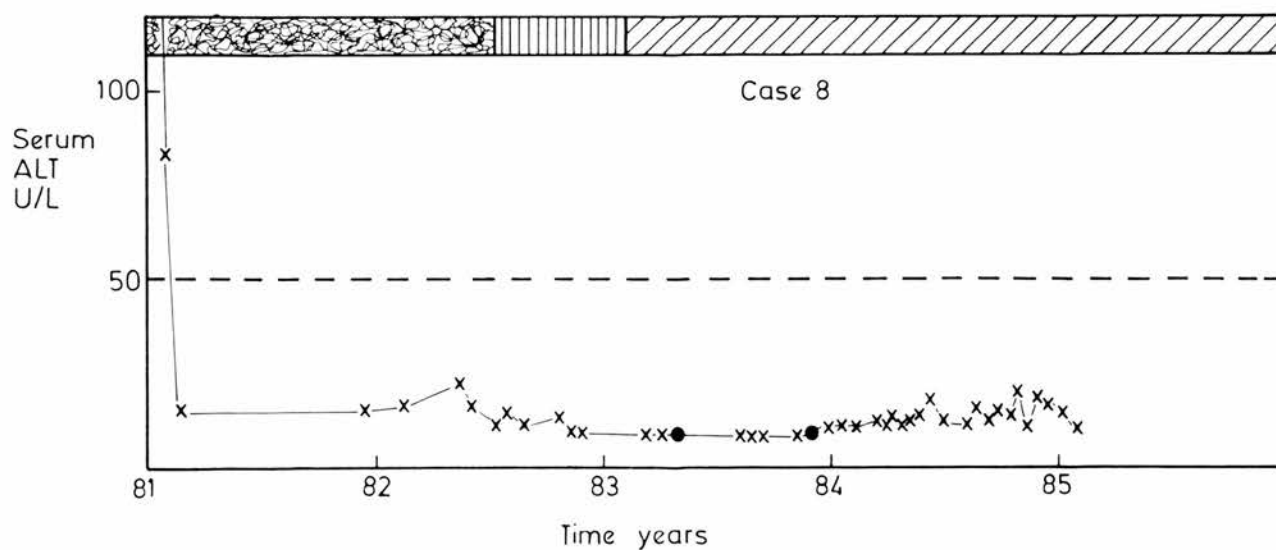
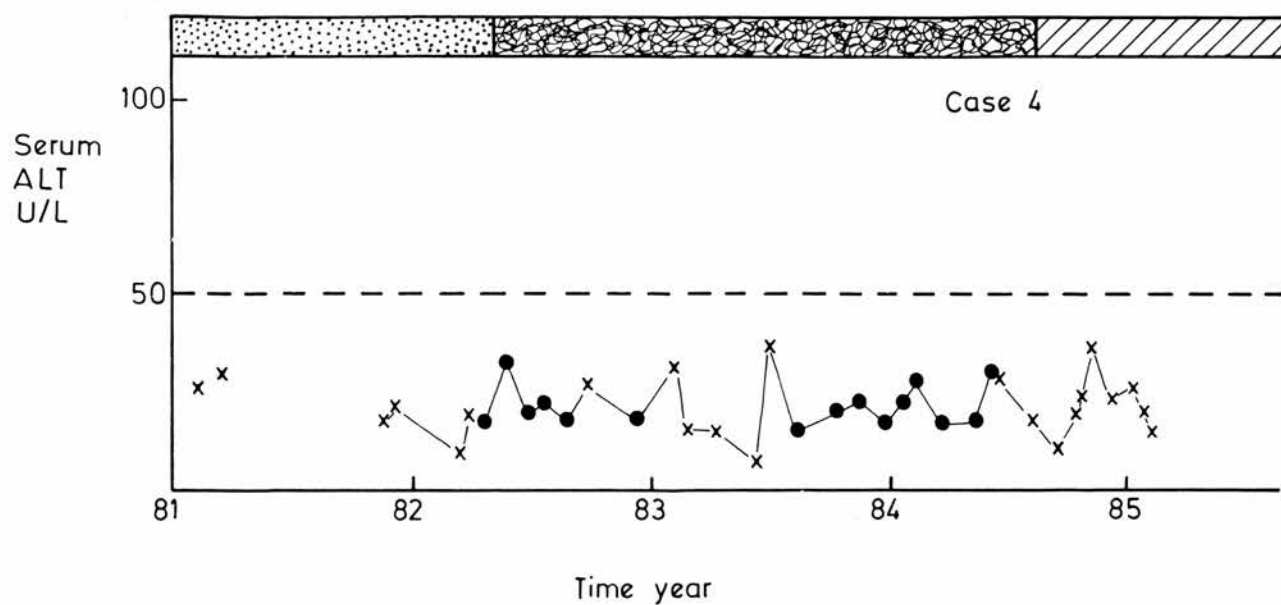
ALT values in seven patients receiving  
different types of immunoglobulin therapy

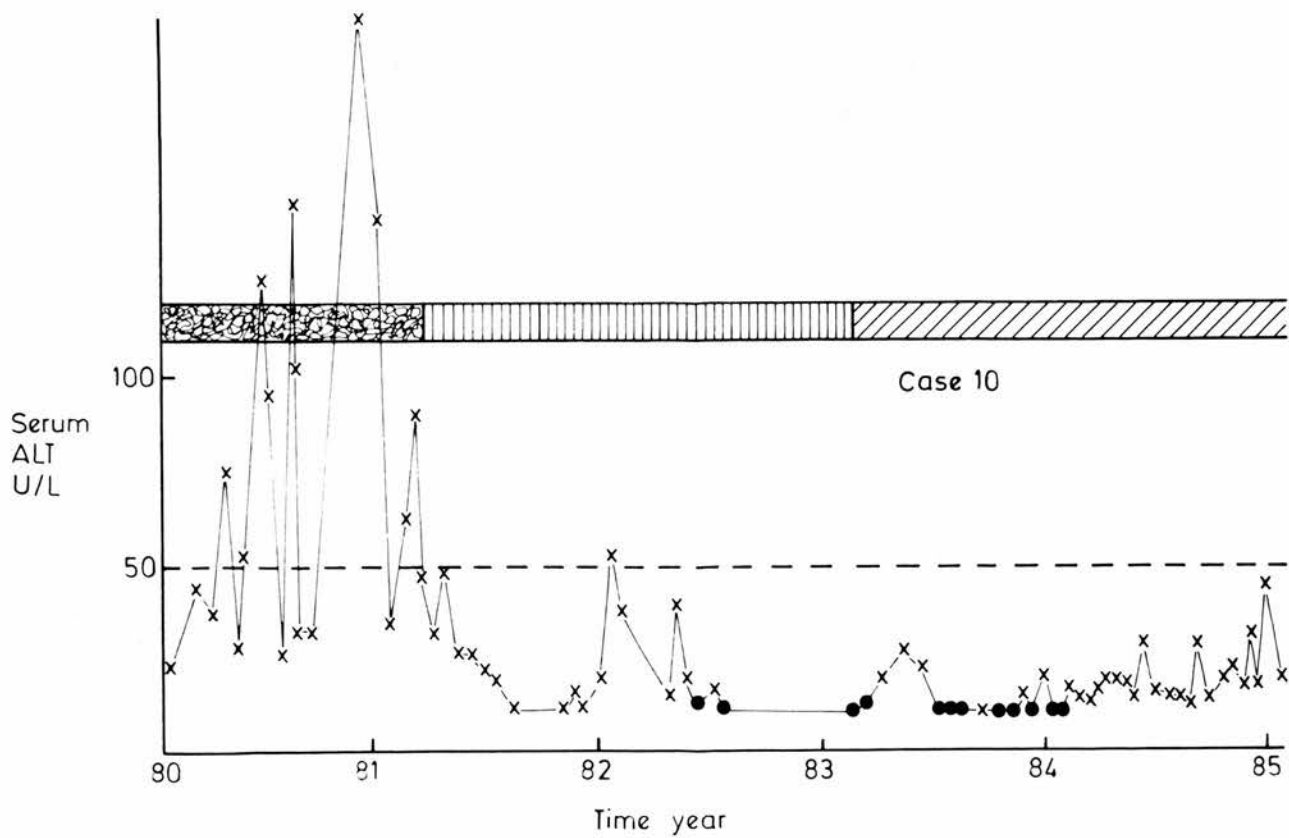
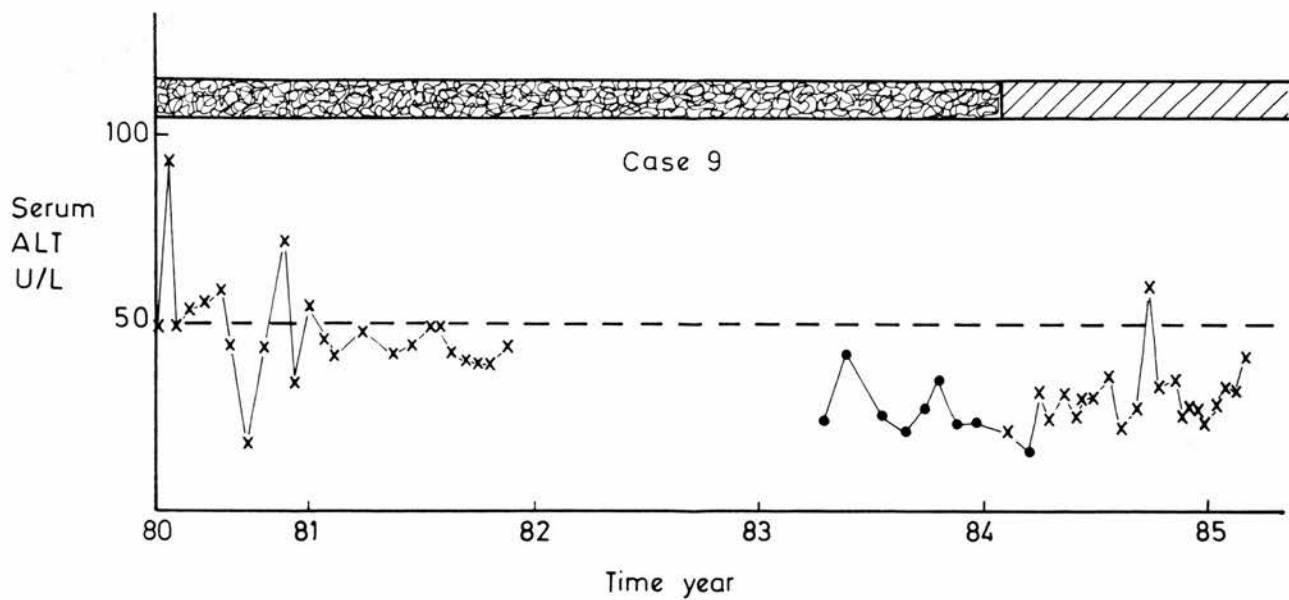
- x = serum ALT values in samples assayed within 72 hours  
of collection
- = serum ALT values in samples assayed after storage  
at -150 C

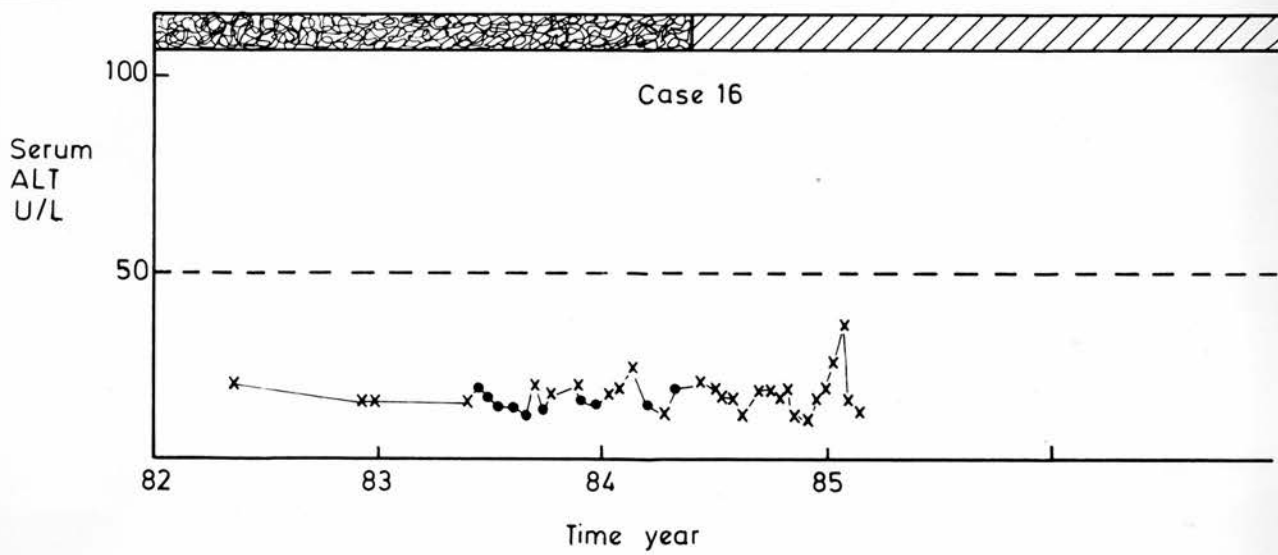
Broken horizontal line indicates the upper limit of  
normal range for ALT levels

Hatched horizontal areas indicate the type of  
immunoglobulin therapy received by the patient  
(Key as in Figure 5.3.1)









infusion. All but seven were in the normal range (10-50 U/l). The seven samples with abnormal values were from four patients ; Case 3 (91 and 53 U/l), Case 9 (60 U/l), Case 15 (70 U/l) and Case 17 (96, 84 and 234 U/l).

An additional 60 serum samples were retrieved from -150 C storage and examined for ALT activity. Only four were abnormal, two from Case 1 (54 and 69 U/l), and two from Case 5 (75 and 58 U/l).

A further five normal ALT values were noted on review of the case notes.

Therefore, of a total of 314 serum ALT values, 11 (from six patients) were above the upper limit of normal (50 U/l).

#### 5.3.5.4 Hepatitis B Serology

HBsAg was negative in all the specimens tested.

#### 5.3.5.5 Relationship of Raised ALT Values to Clinical Condition of Patients

##### 5.3.5.5.1 FFP therapy

Three out of eight patients studied developed raised serum ALT values during FFP therapy that could be consistent with NANB hepatitis. However, in each case there were more probable explanations for the abnormal ALT. One patient (Case 1) was diagnosed as suffering from sarcoidosis which was active at the time the ALT was raised (Leen et al, 1985b). Another patient (Case

10) was clinically unwell with recurrent episodes of septicaemia and had a cholestatic pattern of liver function tests (alkaline phosphatase of 3351 U/l). The last patient (Case 9) had multiple problems associated with hypogammaglobulinaemia including severe malabsorption with weight loss and recurrent chest infections.

#### 5.3.5.5.2 SNBTS IV IgG

From a total of 314 blood samples, 11 abnormally raised ALT values were observed occurring in six of the 16 patients during therapy with SNBTS IV IgG. No markers of HBV infection were detected in the patients at any time.

Figures 5.3.4c, 5.3.4d and 5.3.4g illustrate three patients with normal serum ALT levels during therapy with FFP and SNBTS IV IgG (Cases 4, 8 and 16).

Further details of the six patients (Cases 1, 3, 5, 9, 15, and 17) who developed raised ALT levels are shown below.

Case 1 had raised serum ALT levels during FFP therapy (see above), secondary to active sarcoidosis and it is likely that the two raised serum ALT values (54 and 69 U/l) were the result of his sarcoidosis.

Case 3 had received SNBTS IV IgG since April 1983. In April 1984, before her 18th infusion of SNBTS IV IgG, she was found to have a high serum ALT of 91 U/l while



remaining clinically very well. Three weeks later her serum ALT was 44 U/l. No cause for the raised ALT was found and in particular there was no history of drug or alcohol ingestion HBsAg, as well as other virological markers of hepatitis, were absent.

Case 5 received Sandoglobulin and Gamimune for three months each prior to the study period. His hypogammaglobulinaemia is associated with malabsorption secondary to villous atrophy, frequent respiratory and gastrointestinal infections requiring treatment with numerous antibiotics including erythromycin for intractable campylobacter enteritis. Two abnormal ALT values (75 and 58 U/l) were observed when the frozen samples were analysed. Retrospective analysis of the case records suggest that he was not unwell at the time of the abnormal ALT levels and there was no apparent cause for the abnormal ALT.

Case 9 had documented abnormal serum ALT values during therapy with FFP. At the time of the single abnormal ALT level (60 U/l) which followed his 15th SNBTS IV IgG infusion, he was asymptomatic.

Case 15 was diagnosed in 1966. After years of treatment with IM gammaglobulin, he received FFP followed by Sandoglobulin and Gamimune (for three months each) before treatment was commenced with SNBTS IV IgG in April 1983. A single raised ALT value (70 U/l) was noted in February 1984. Four days elapsed between the

collection of the sample and the separation of the serum, and all subsequent serial ALT values have been normal. An additional factor in this case is that the attending physician reports that this patient has a large regular alcohol consumption.

Case 17 had been on total parenteral nutrition since 1980 because of intractable diarrhoea with severe malabsorption. His medical history included chronic cholecystitis and cholelithiasis treated by cholecystectomy in 1980, and since 1981 documented abnormal liver function tests including raised ALT ranging from 50 to 234 U/l. A liver biopsy in 1980 showed mild chronic portal triaditis with occasional granulomatous foci. A repeat liver biopsy in 1984 had similar features, and in particular there were no features to suggest NANB hepatitis. His abnormal liver function tests were considered by his gastroenterologist to be a consequence of his underlying disorder and of an excess intralipid content in his total parenteral nutrition regime (Sheldon et al, 1978; Lindor et al, 1979).

#### 5.3.6 Discussion

Many serum enzymes may remain stable for some time when stored at -20 C. Serum ALT has also been shown to be stable for three to four days at room temperature and up to seven days if kept refrigerated at 4 C

(Wilding et al, 1977; Schmidt et al 1976). The data presented here suggests that while there seems to be a fall in serum ALT activity after storage at -150 C for six months, the fall is minimal in contrast to storage at -20 C (Figure 5.3.2). It also seems unlikely that any serum sample with significantly raised ALT values (100 U/l or greater when considering hepatitis) would have been missed because they had been stored at -150 C for six months.

Three of the patients receiving FFP have been noted to have raised serum ALT levels. Although the magnitude of their serum ALT rise is consistent with hepatitis there has been an obvious cause for their abnormally raised ALT; one had active sarcoidosis, and the remaining two were very ill with infection including recurrent septicaemia in one patient. In addition, there were no features to suggest NANB hepatitis.

None of the patients during therapy with SNBTS IV IgG would fulfil the criteria for hepatitis except for the patient with known liver disease. Only a small proportion of patients receiving regular IV IgG infusions had any ALT abnormalities. The pattern and magnitude of disturbance of liver function tests was not that of NANB hepatitis. In five of the six patients with raised ALT levels during treatment with IV IgG there appears to be an explanation other than NANB hepatitis. Only one patient had an unexplained elevated ALT levels

(peak ALT of 91 U/l) which became normal within three weeks, but by definition (Stevens et al, 1984) she did not have hepatitis and was clinically well throughout. Furthermore, neither the clinical picture nor the level of serum ALT rise in these patients resemble that of NANB hepatitis or the illness described by Lever et al (1984) and Ochs et al (1985).

Conventional immunoglobulin preparations virtually never transmit viral infections (Finlayson, 1979; Lane, 1983; Iwarson et al, 1985). However, several recent reports (Lever et al, 1984 ; Ochs et al, 1985) indicate that some immunoglobulin products designed for intravenous administration may not be as safe as the conventional intramuscular product. It is probable that production methods influence the safety of intravenous preparations in terms of transmission of viral infections. In particular, the presence or absence of further processing to reduce the level of spontaneous anti-complementary activity and of vasoactive enzymes following the removal of ethanol may be important. Not all of these procedures are thought to be virucidal. Neither of the two intravenous preparations known to have transmitted NANB hepatitis (Lever et al, 1984; Ochs et al, 1985) were subjected to a recognised virucidal finishing treatment, for example, acid treatment (pH 4.0) or beta-propiolactone and ultraviolet irradiation (Prince et al, 1983). It can be concluded

that SNBTS IV IgG which is processed for intravenous use by the pH 4.0/pepsin method (Barandun et al, 1962) and FFP obtained from two to four properly screened dedicated donors do not transmit NANB hepatitis.

## 5.4 HALF-LIFE OF INFUSED IMMUNOGLOBULIN

### 5.4.1 Introduction

Serum IgG levels achieved in individual patients following IgG administration are variable, probably as a consequence of the variable half-life of infused IgG in such patients. The half-life of infused IgG depends primarily upon the intactness of the infused IgG molecule, more specifically on the Fc portion of the IgG molecule; preparations in which this site is digested or altered have a shortened half-life. It is also influenced by other factors including IgG loss through intestinal and bronchial mucosa, which is itself dependent on the degree and chronicity of inflammation affecting those areas.

Knowledge of the half-life of any IV IgG preparation will dictate the frequency of immunoglobulin administration, which up to now has been decided on an empirical basis. An attempt was therefore made to determine the half-life of infused SNBTS IV IgG in patients with primary hypogammaglobulinaemia as part of the evaluation of SNBTS IV IgG.

### 5.4.2 Materials and Methods

Blood was collected before and immediately after each infusion of IV IgG for the measurement of serum IgG levels. Blood samples for half-life estimations were collected from seven patients, in most cases after their

first infusion of IV IgG, and additional sequential blood samples were collected at different intervals until the next infusion, usually 15 to 30 days later.

Total serum IgG levels were measured in the sequential samples from seven patients, and serum CMV IgG levels in four patients; the log serum values were plotted against time.

Serum IgG measurements were performed by Mr G Neill and his technicians at the Edinburgh Blood Transfusion Centre using laser nephelometry (Whicher et al, 1978). the normal range for serum IgG concentrations (5.0 to 13.0 g/l) was evaluated by the measurement of IgG levels in 350 normal blood donors.

Serum CMV IgG levels were measured by Dr B Cuthbertson, of the Protein Fraction Centre of the SNBTS, using an ELISA system at a single dilution in duplicate. A complement fixing CMV antigen (Flow Laboratories) was coated on the plates and the samples compared to serial dilutions of a reference plasma standard (Salonem and Vaheri, 1981).

The half-life of the infused IV IgG was determined by assuming that the metabolism of IgG follows first order kinetics, as reported by Waldmann and Strober (1969). Briefly, there is an early alpha-phase, which represents redistribution of the IgG, followed by a slower beta-phase, which represents the metabolism of IgG (Figure 5.4.1). The half-life is proportional to the slope of

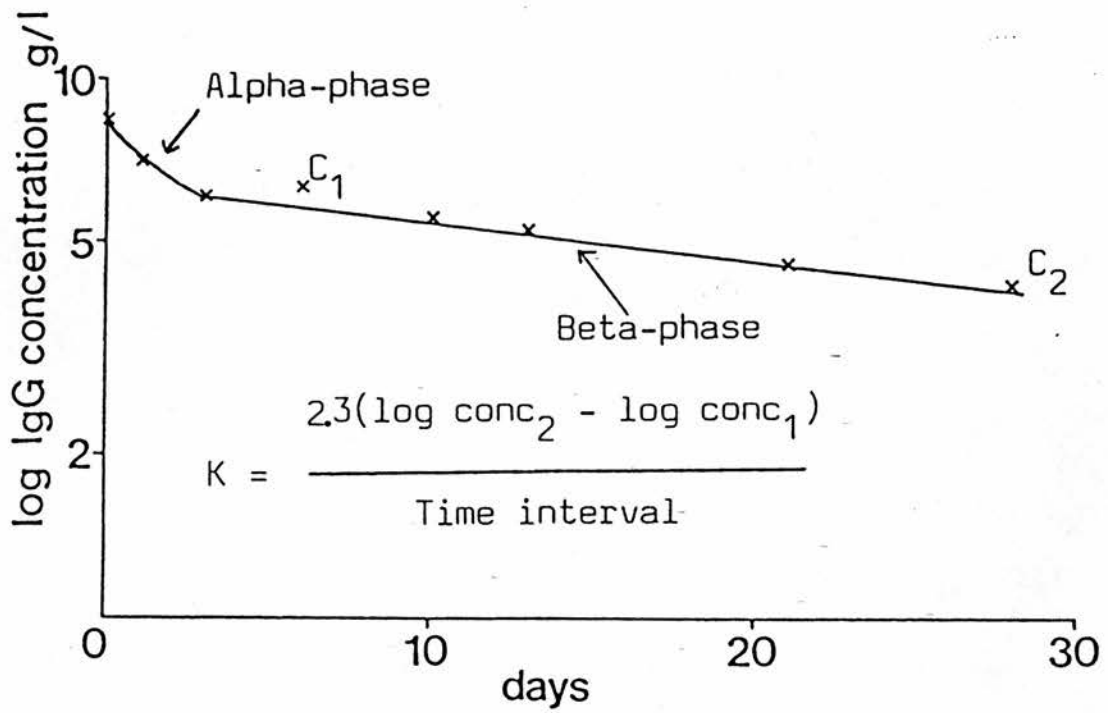


Figure 5.4.1

Measurement of half-life of infused IgG

Horizontal axis : time in days

Vertical axis : log IgG concentration



the beta-phase :

$$\text{Half-life} = \log 2/k = 0.693/k$$

where  $k$  is the slope of the beta-phase determined by linear regression analysis.

#### 5.4.3 Results

The results have been plotted in Figures 5.4.2 to 5.4.12. Half-life values of total IgG ranged from 15 to 53 days and half-life values of CMV IgG ranged from 35 to 61 days.

#### 5.4.4 Discussion

The half-life values are prolonged and consistent with those obtained in other patients with primary hypogammaglobulinaemia. Indeed, just as in the study by Schiff (1985), the serum IgG of the patient at the time of the study did not influence the half-life of infused IgG. This observation is, however, not consistent with the previous study by Waldmann and Strober (1969), who reported that patients with higher immunoglobulin levels seemed to have the shortest half-lives.

Specific antibody levels have been used in this study to measure the half-life of infused IgG, to avoid any possible artifact introduced by intrinsic IgG production. Because the number of patients was small, no comments can be made regarding the difference in the half-life values estimated from total serum IgG levels

Figures 5.4.2 - 5.4.8

(Cases 1-7)

Half-life of infused IgG using  
total IgG measurement

Horizontal axis : time in days

Vertical axis : log IgG concentration

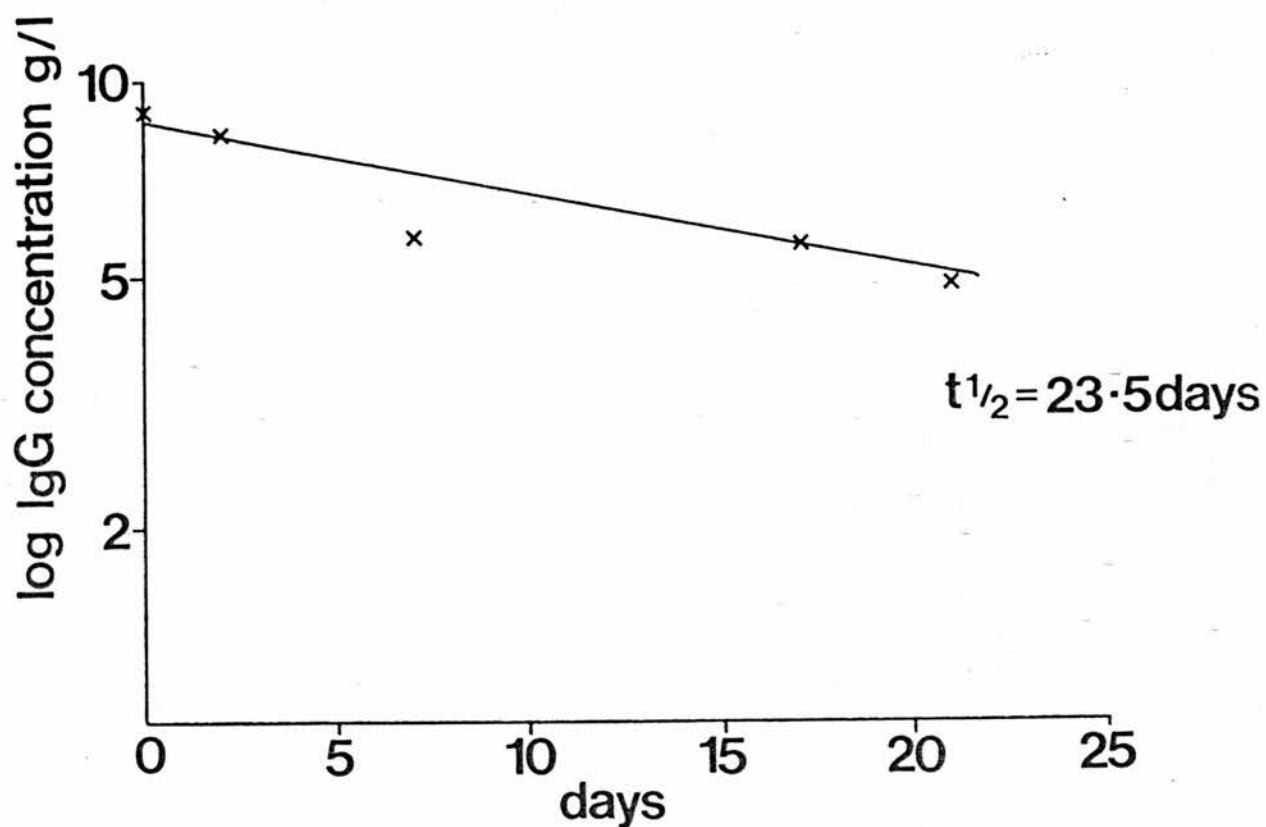


Figure 5.4.2

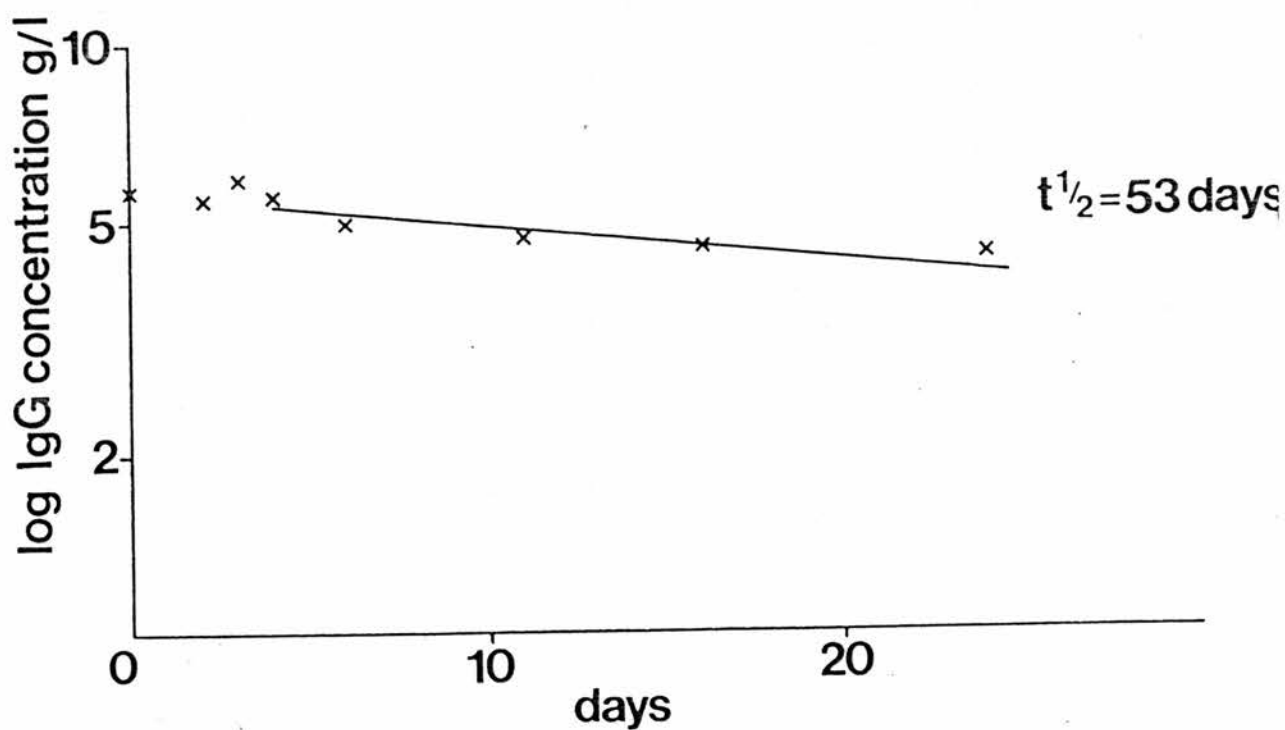


Figure 5.4.3

Figure 5.4.4

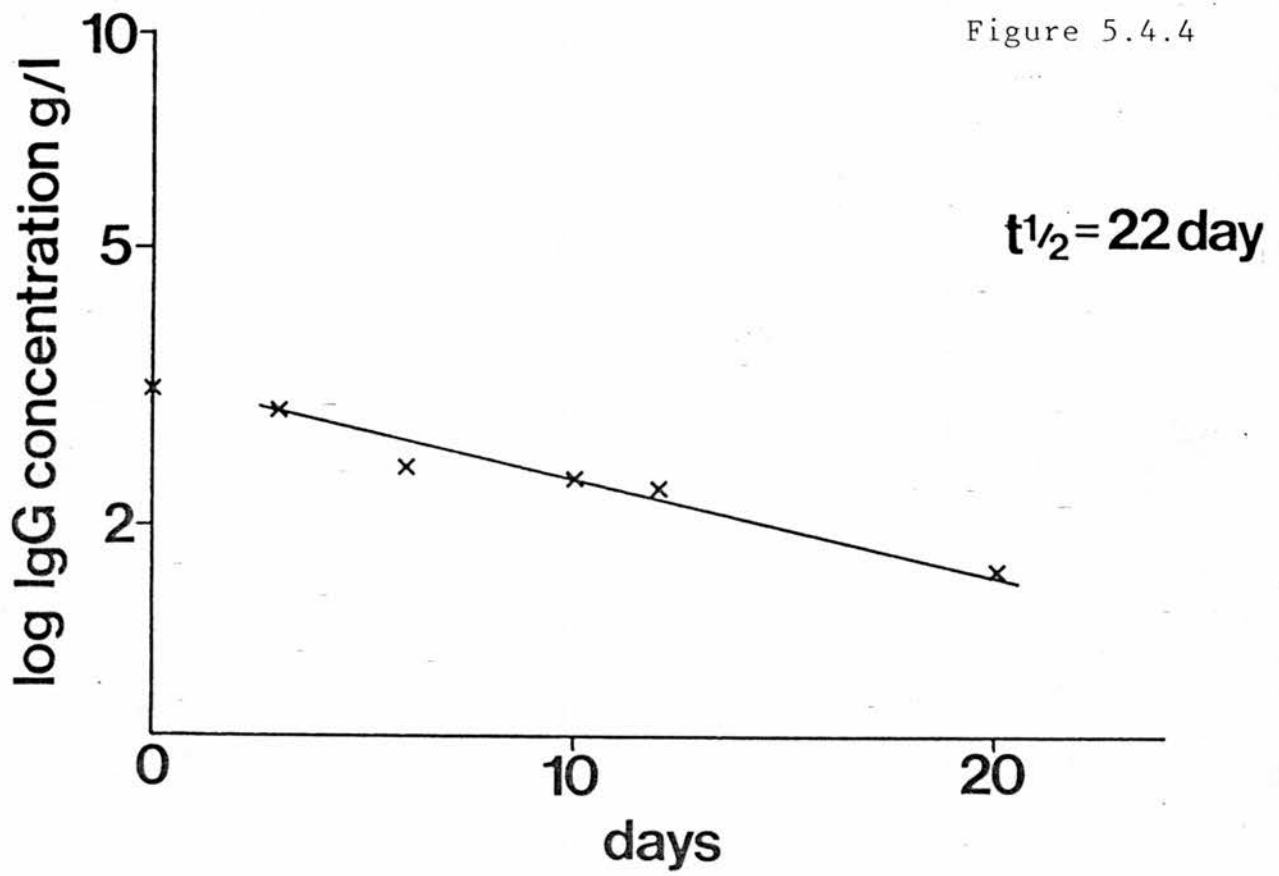


Figure 5.4.5

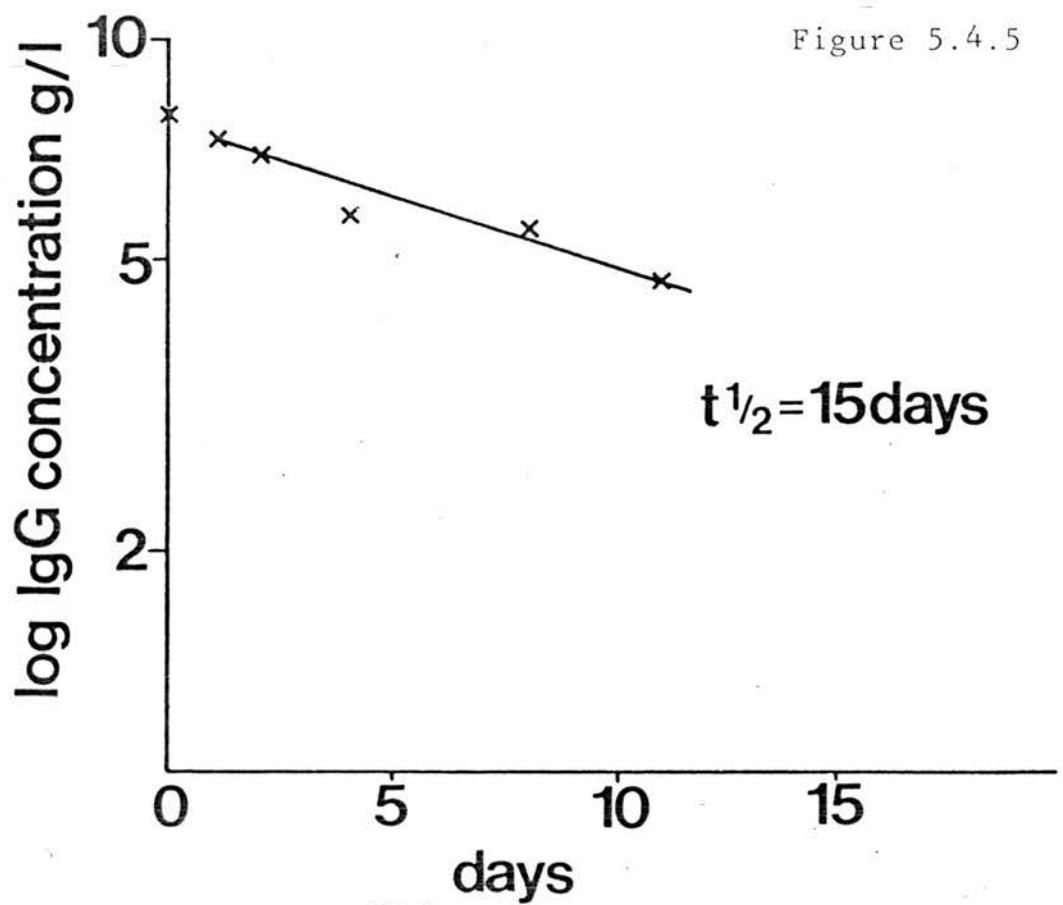


Figure 5.4.6

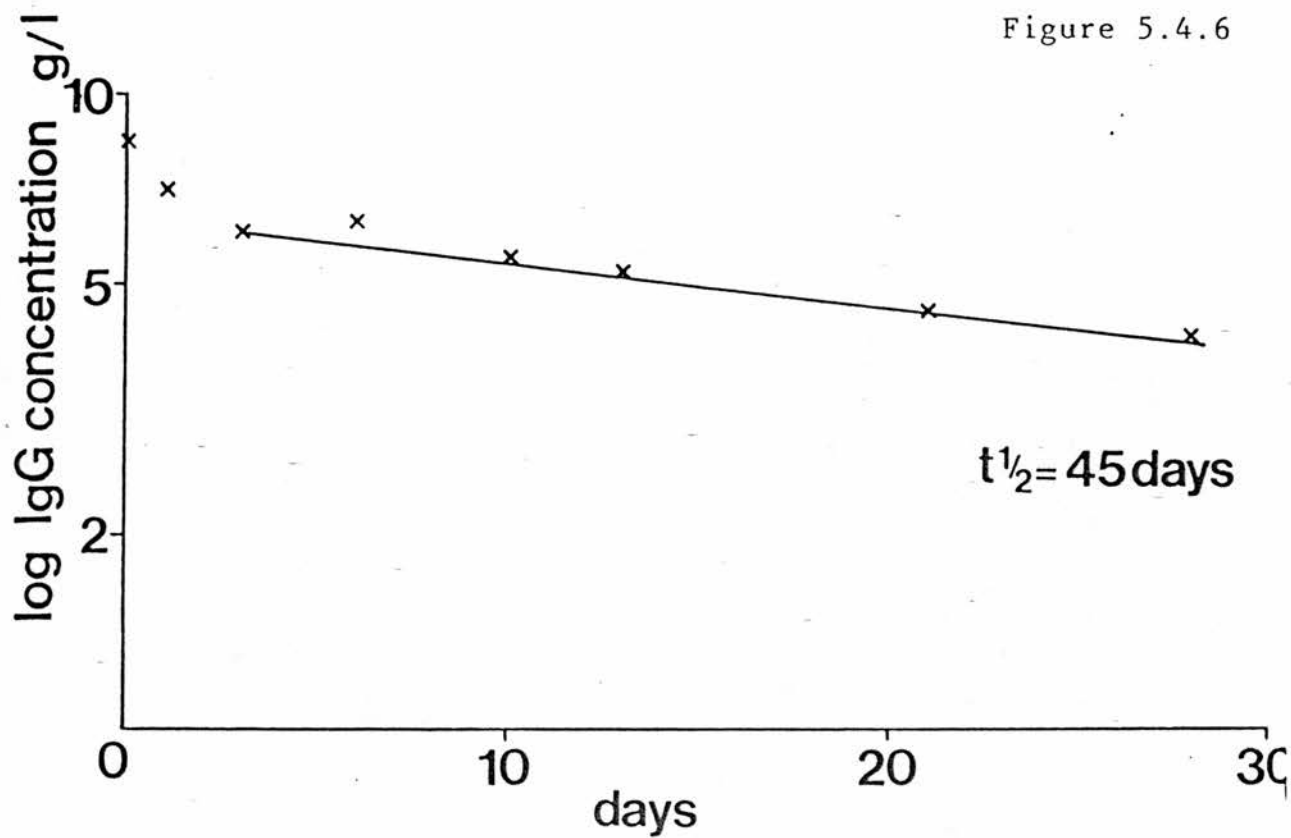
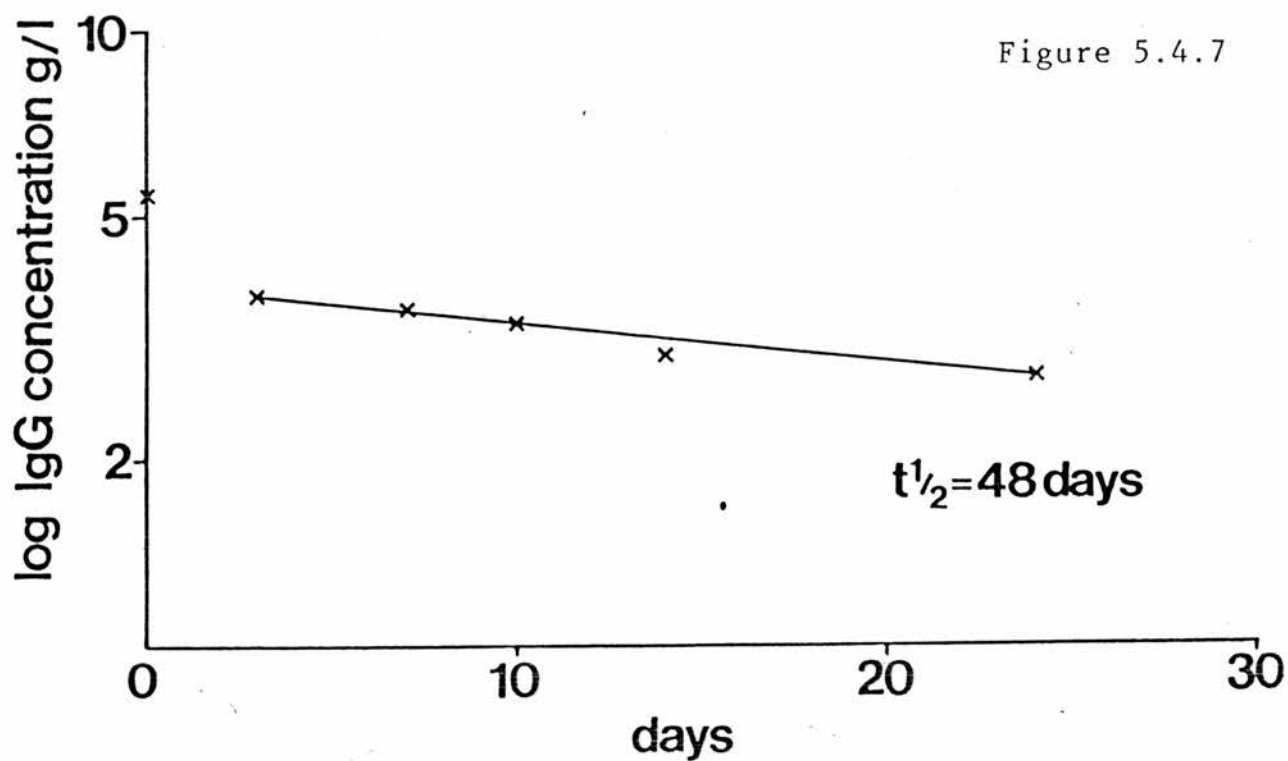


Figure 5.4.7



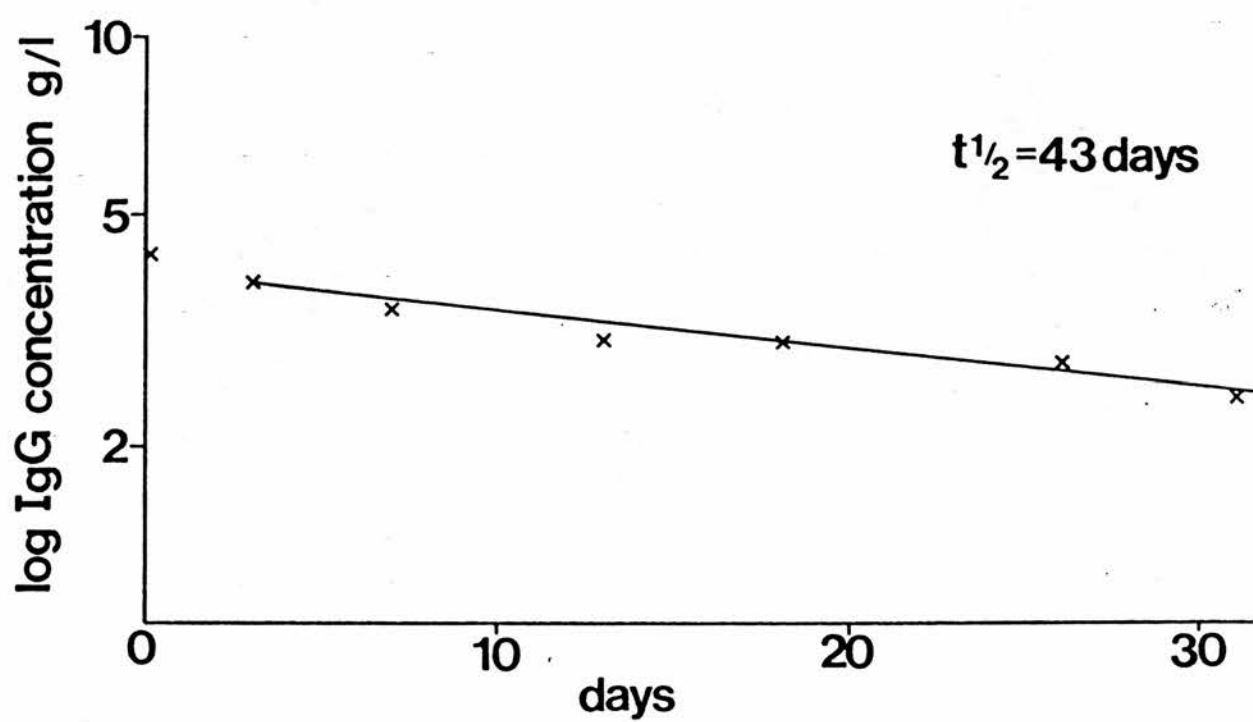


Figure 5.4.8

Figures 5.4.9 - 5.4.12

(Cases 2,3,5 and 6)

Half-life of infused IgG using  
CMV IgG assay

Horizontal axis : time in days

Vertical axis : log CMV IgG level

Figure 5.4.9

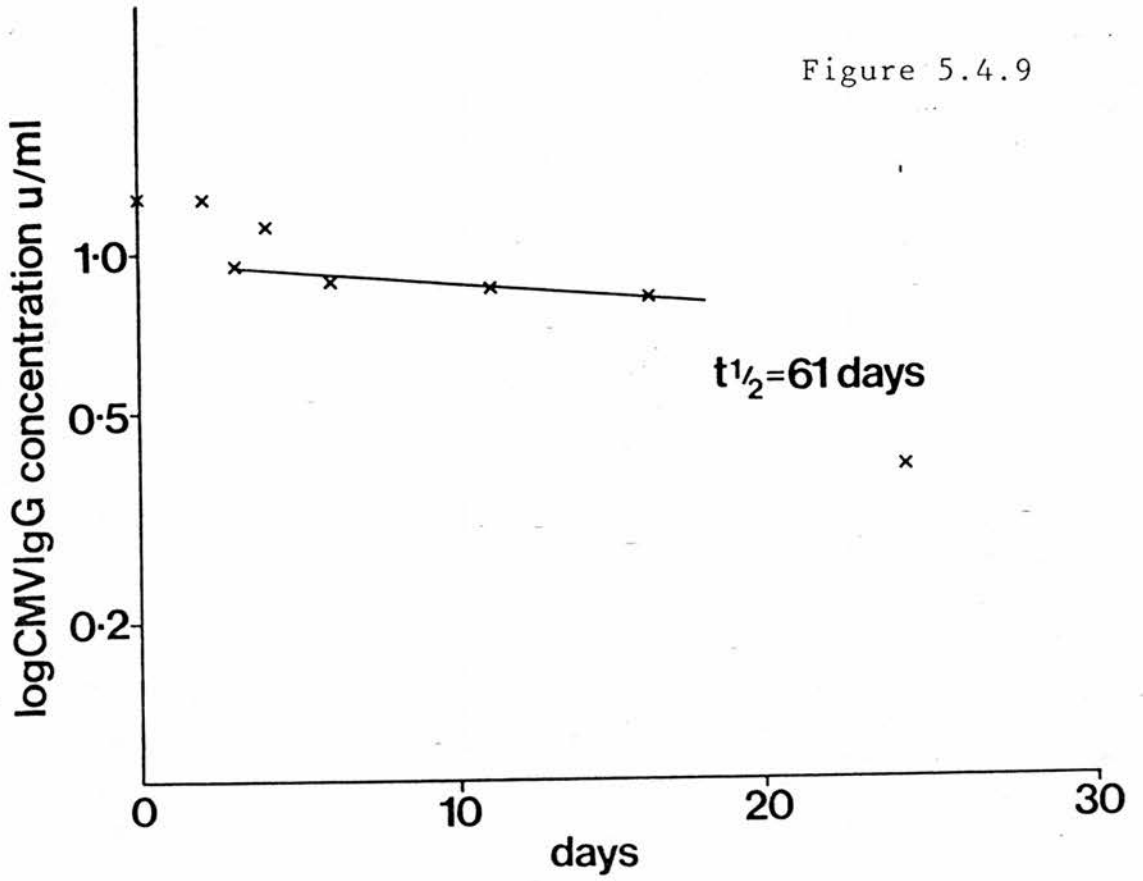


Figure 5.4.10

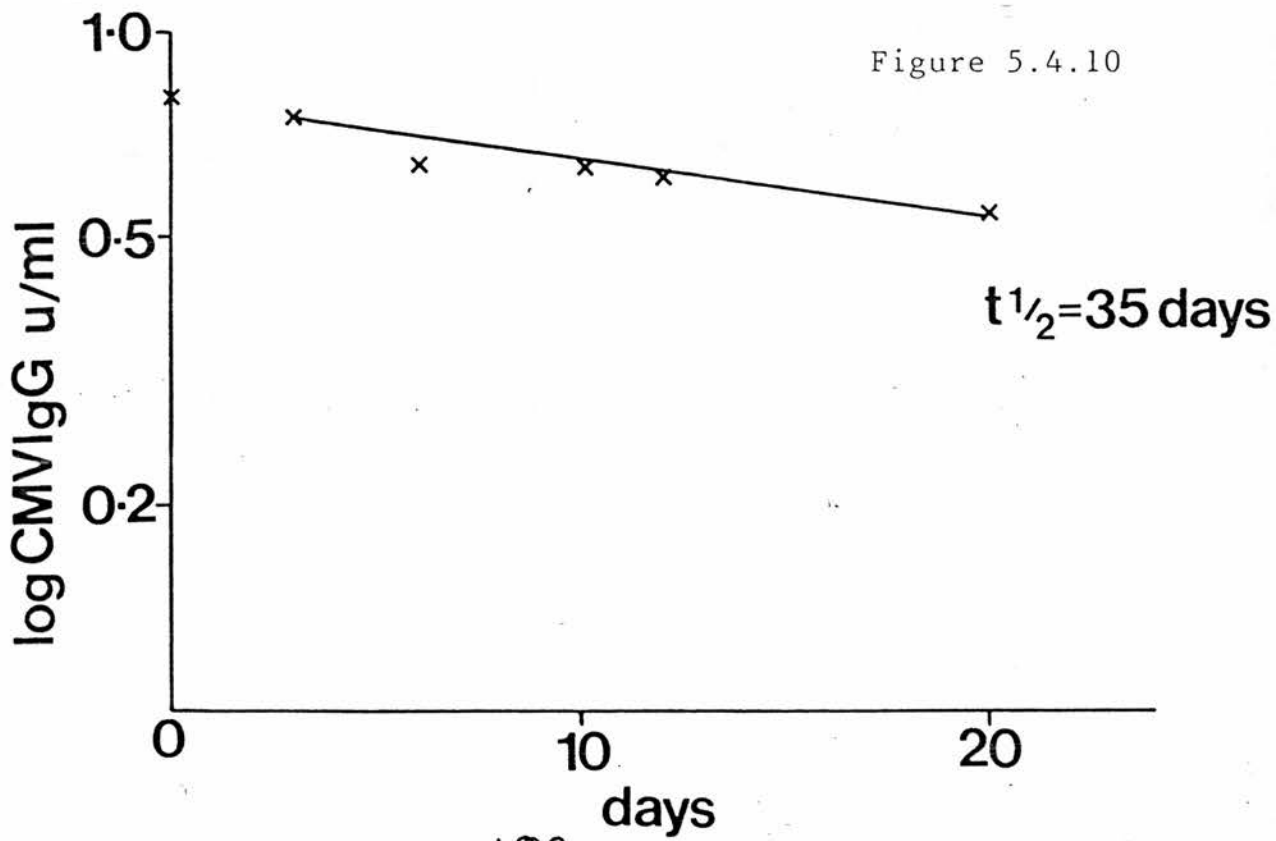




Figure 5.4.11

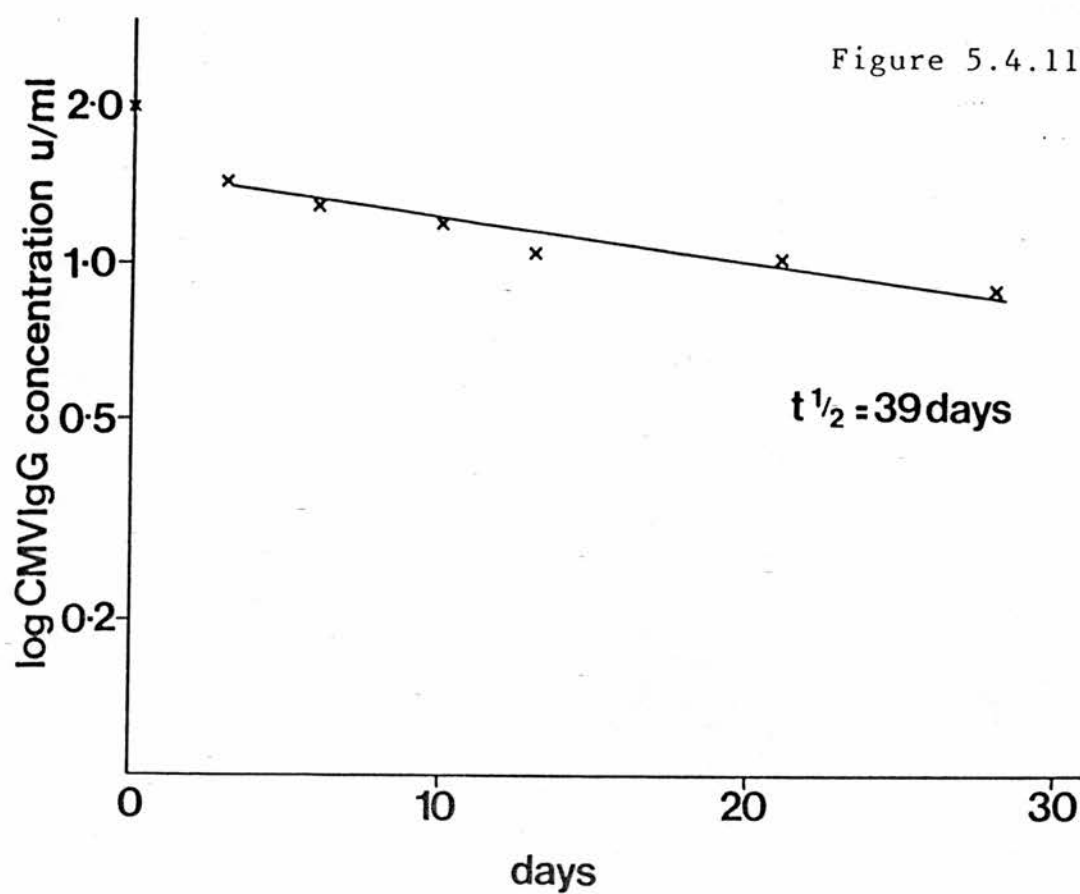
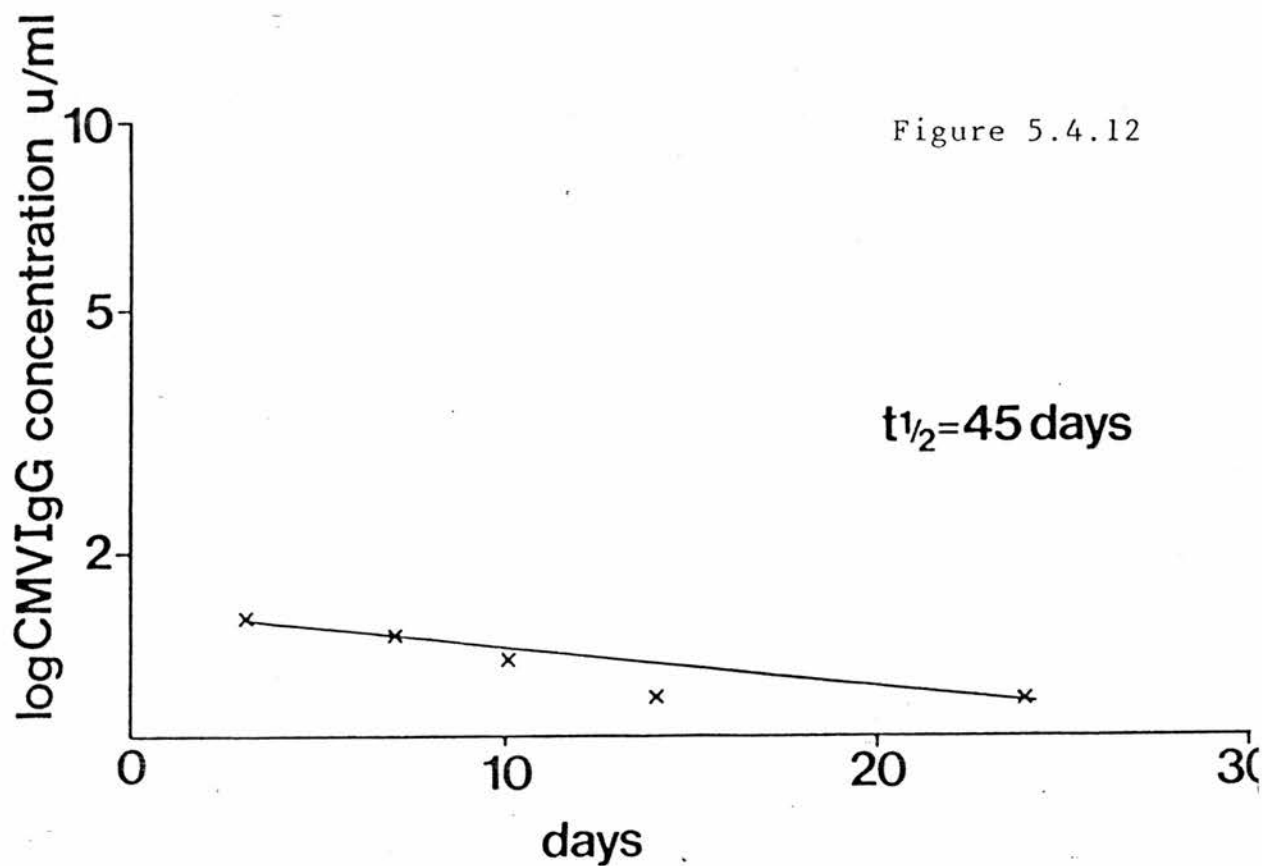


Figure 5.4.12



and from serum CMV IgG levels.

No attempt was made to repeat the half-life measurements after a prolonged period of treatment. However, there is some evidence that the half-life of infused IgG following a nine-month course of IV IgG was longer than at the start of therapy (Lever et al, 1987)

Intravenous immunoglobulin manufactured by the mild pepsin treatment at pH 4.0 therefore has an acceptable half-life.

## 5.5 SERUM IMMUNOGLOBULIN LEVELS IN PATIENTS WITH PRIMARY HYPOGAMMAGLOBULINAEMIA DURING SNBTS IV IgG THERAPY

### 5.5.1 Introduction

The dose of IgG administered to patients with hypogammaglobulinaemia (25-50 mg/kg/week) has been based on the clinical trial run by the MRC (1971). However, the increments in serum IgG level achieved in patients treated with this dose of IgG are highly variable and IgG levels hardly ever rise into the normal range. The intravenous administration of large quantities of immunoglobulin has now been shown to be both safe and well-tolerated in patients with primary hypogammaglobulinaemia (see section 5.2), confirming the earlier findings of Barandun and Morell (1981), Cunningham-Rundles et al (1981) and Wedgwood and Ochs (1981). As a result, clinicians have attempted to achieve higher serum IgG concentration in their patients with a view to reducing the incidence of infections. Most trials have used larger doses of IgG per infusion but the intervals between infusions have been kept fixed at either three or four weeks (Cunningham-Rundles et al, 1984; Eibl et al, 1984; Schiff et al, 1984). The optimal dose schedule has therefore not been established.

The objective of this study was to examine whether normal or near normal 'trough' serum IgG concentrations could be achieved in patients with primary

hypogammaglobulinaemia with individualised doses of infused IgG. The effects of varying either the dose of IgG infused or the frequency of infusions, or of varying both the dose and frequency of IgG infusions, are discussed in this section.

#### 5.5.2 Patients, Materials and Methods

##### 5.5.2.1 Patients

A total of 17 patients (age range 11 to 50 years ; 11 male and six female ; see Table 5.1.2) received SNBTS IV IgG for at least six months between February 1983 and May 1985. The duration of treatment was over 12 months in 13 of the 17 patients, over 18 months in 10 patients and over two years in eight patients. Twelve suffered from late onset primary hypogammaglobulinaemia (CVI) and five from X-linked primary hypogammaglobulinaemia. All patients had a history of recurrent bacterial infections, with low serum IgG levels measured on at least two occasions at the time of diagnosis. All but two were already established on immunoglobulin replacement therapy before entry into the study. Further clinical details of individual patients are shown in Table 5.5.1.

Consent was obtained from the local hospital Ethics Committee, the patient's own physician and from the patient prior to the commencement of the study.

Case No	Age at start of study (years)	Diagnosis	Other diagnoses*	Clinical outcome** (improvement)	Chest	GI	IgG at diagnosis g/l	IgG before study g/l	IgG after study g/l	Treatment during 3 months before study
1	29	CVI	IDDM	Yes	Yes	n/a	2.9	5.47	5.28	FFP 1 litre/3 weeks
2	21	CVI	Sarcoidosis	Yes	Yes	No	1.49	2.5	5.23	IM IgG 1.5g/week
3	25	CVI	Giardiasis	Yes	Yes	n/a	1.0	4.34	8.4	IV IgG Gamimune 0.2g/kg/3 weeks
4	22	CVI	Meningitis							
5	29	CVI	Bronchiectasis	No	Yes	n/a	2.3	1.53	4.7	Nil
6	41	CVI	Villous atrophy	Yes	Yes	No	3.0	8.08	4.40	FFP 800-1500ml/3 weeks
7	29	CVI	Ileal strictures	Yes	Yes	No	1.1	2.73	7.2	IV Gamimune 0.2g/kg/3 weeks
8	41	CVI	Sarcoidosis	Yes	Yes	n/a	0.7	6.24	3.8	IV Gamimune 0.2g/kg/3 weeks
9	19	X-linked	NLH	Yes	Yes	n/a	1.7	0.97	4.05	IM IgG 750mg/month
10	16	X-linked	Bronchiectasis	Yes	Yes	No	undetectable	1.46	3.05	IV Sandoglobulin 0.3g/kg/2 weeks
11	11	X-linked	Villous atrophy	Yes	Yes	No	1.5	3.22	2.8	FFP 400ml every 3 weeks
			Hypoparathyroidism							
			Renal artery aneurysms	Yes	Yes	n/a	undetectable	2.24	4.7	IV Sandoglobulin 0.2g/kg/2 weeks
12	21	CVI	Bell's palsy	Yes	Yes	n/a	2.3	3.36	4.8	IV Gamimune 0.2g/kg/3 weeks
13	37	CVI	Giardiasis	Yes	Yes	No	-	1.57	4.78	IM IgG 1.5g every 10 days
			Varicella Zoster							
14	50	CVI		Yes	Yes	n/a	1.8	1.8	4.4	Nil
15	23	X-linked	Bronchiectasis	Yes	Yes	n/a	-	3.92	3.53	IV Gamimune 0.2g/kg/3 weeks
16	19	X-linked	Epilepsy	No	No	n/a	-	6.06	2.7	FFP 600ml every 2 weeks
			Measles							
			Encephalitis							
			Malabsorption							
			Gall stones							
17	41	CVI	Total parenteral nutrition	Yes	Yes	n/a	2.6	2.86	4.2	IM IgG 750mg weekly

\* All patients had a history of recurrent upper respiratory tract infections before the commencement of immunoglobulin replacement therapy.

\*\* Clinical outcome of IV IgG therapy, defined as improvement in either respiratory or gastrointestinal symptoms compared with symptoms while on IM IgG therapy or on no IgG replacement therapy (n/a = evaluation not applicable as patient did not have gastrointestinal symptoms)

Table 5.5.1 - Details of individual patients and their response to therapy

### 5.5.3 Study design

#### 5.5.3.1 Infusion Dose and Rate

A total SNBTS IV IgG dose of 200 mg/kg body weight was administered on most occasions. In two patients, cases 9 and 10, where the 'trough' serum level, i.e. immediately prior to IgG infusion, was not maintained above 3 g/l, the infusion dose was steadily increased to 540 and 400 mg/kg respectively.

In most patients the infusion was administered at a rate of 120 mg/kg/hr. In patients who had never received any immunoglobulin replacement, this rate was initially reduced in the first infusions. The infusion rate was increased to 264 and 240 mg/kg/hr for cases 9 and 10 without any adverse reactions.

#### 5.5.3.2 Frequency of Infusion

Infusions were initially administered at three-weekly intervals, except in three patients (cases 9, 10 and 11) who received their infusions at two-weekly intervals. Infusion frequency was modified according to the patient's clinical state while aiming to maintain a serum 'trough' IgG level as near to the lower limit of normal as possible.

The infusion frequency was reduced from three-weekly to four-weekly (or even less) if the 'trough' serum IgG was above 4 g/l (normal range 5.0 to 13.0 g/l), and if the patient remained free from infection. If, however,

the patient was clinically unwell and the 'trough' serum IgG was below 3 g/l, the infusion frequency was increased to two-weekly intervals. If the 'trough' serum IgG was still below 3 g/l the infusion dose was doubled or given at weekly intervals. Finally, if there was still no response a doubled infusion dose was administered at weekly intervals. The frequency of infusion therefore ranged from one week (cases 6 and 9) to 12 weeks (case 3).

A total of 517 infusions from 24 batches of SNBTS IV IgG was therefore administered to 17 patients over the study period.

#### 5.5.3.3 Methods of Assessment

Venous blood samples were collected from the infusion site at the commencement of the infusion and at the end of the infusion, either from the opposite arm or from the infusion site, after it had been flushed with 10 ml of blood. Serum IgG levels were assayed in the pre- and post-infusion samples.

IgG was measured by laser nephelometry as described in section 5.4.2 (Whicher et al, 1978).

#### 5.5.4 Results

##### 5.5.4.1 Serum IgG Concentrations Following SNBTS IV IgG Therapy

###### 5.5.4.1.1 Sequential serum IgG concentrations

Figures 5.5.1 to 5.5.6 demonstrate sequential serum IgG levels before and after each infusion in selected patients. Pre-infusion samples give an indication of the 'trough' level or lowest serum IgG level following SNBTS IV IgG infusion, while post-infusion samples collected immediately following the immunoglobulin infusion are a reflection of the 'highest' level that might be achieved following SNBTS IV IgG infusion.

In 13 of 17 patients who had received SNBTS IV IgG for more than six months, the serum 'trough' IgG level was above 4.0 g/l within eight months of the commencement of SNBTS IV IgG replacement therapy. In three of four patients who had received IM IgG before entry into the study, the 'trough' serum IgG level was higher than 4.0 g/l within three months of their initial infusion. Of the two patients who had never received any form of replacement therapy previously, only one achieved a trough IgG concentration greater than 4 g/l with the first three months but both achieved that level within eight months from the first infusion.

Six of the 17 had a serum 'trough' IgG level of



greater than 5.0 g/l within six months of treatment. Of the 10 patients who received treatment for at least 18 months, half of them maintained a 'trough' serum IgG level of greater than 5.0 g/l for at least a year while 80% maintained their 'trough' IgG above 4.0 g/l for at least 12 months. Seven maintained the 'trough' IgG above 5.0 g/l for more than three months and eight for more than two months.

Seven patients started treatment with SNBTS IV IgG in early 1983, and their sequential serum IgG levels show the cumulative effect of IV IgG therapy, as shown in Case 11 (Figure 5.5.5). Two patients with bronchiectasis maintained their serum IgG level within the normal range. Some of the high serum IgG levels prior to commencing SNBTS IV IgG therapy were due to preceding therapy with FFP or another intravenous immunoglobulin preparation (e.g. Case 5, Table 5.5.1).

#### 5.5.4.1.2 Effect of increasing dose

Two patients (Cases 9 and 10) with X-linked primary hypogammaglobulinaemia who had persistently low serum IgG levels, and who could not attend for infusions more often than once every two weeks, had their infusion dose increased to 540 and 400 mg/kg. An increment in the serum IgG level was observed in only one patient (Case 10, Figure 5.5.4). Attempts at further increasing the serum IgG level in Case 9 consisted of

Figures 5.5.1 - 5.5.6

Serial serum IgG levels in six patients  
on SNBTS IV IgG replacement

The date of serum IgG measurement is shown on the horizontal axis and serum IgG concentration on the vertical axis. The lower limit of the normal range for serum IgG concentrations (5 g/l) is shown by a horizontal broken line and the infusion dose and frequency of SNBTS IV IgG infusion is shown at the top of each Figure.

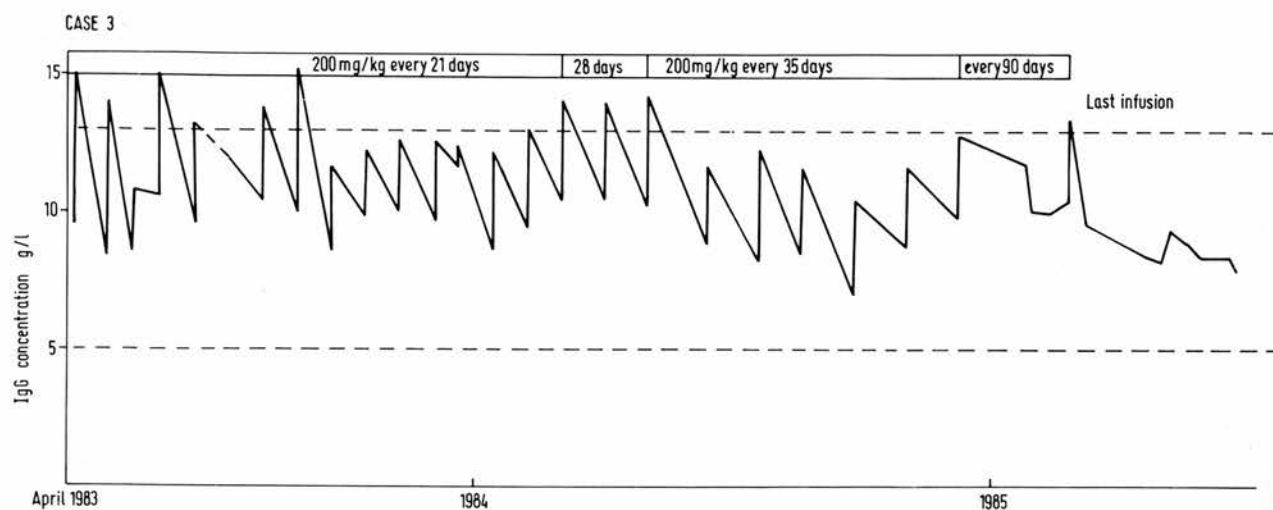


Figure 5.5.1

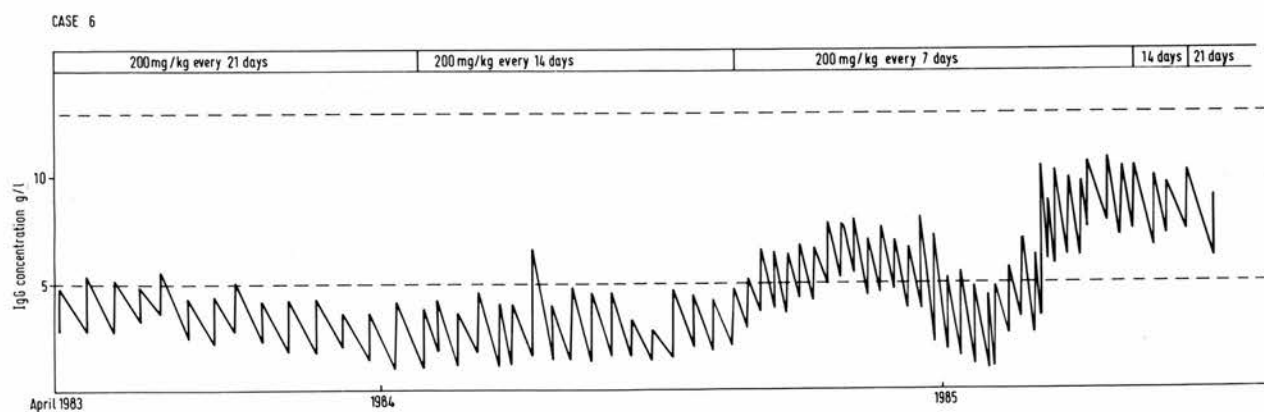


Figure 5.5.2

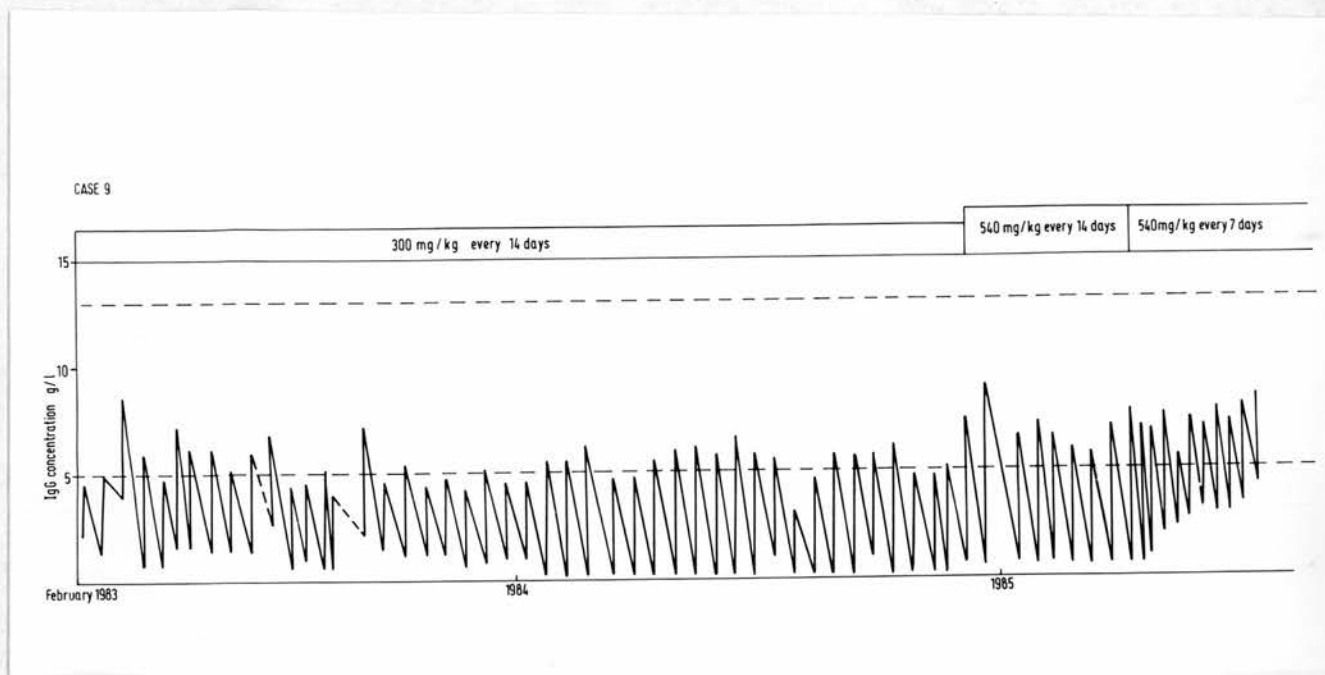


Figure 5.5.3

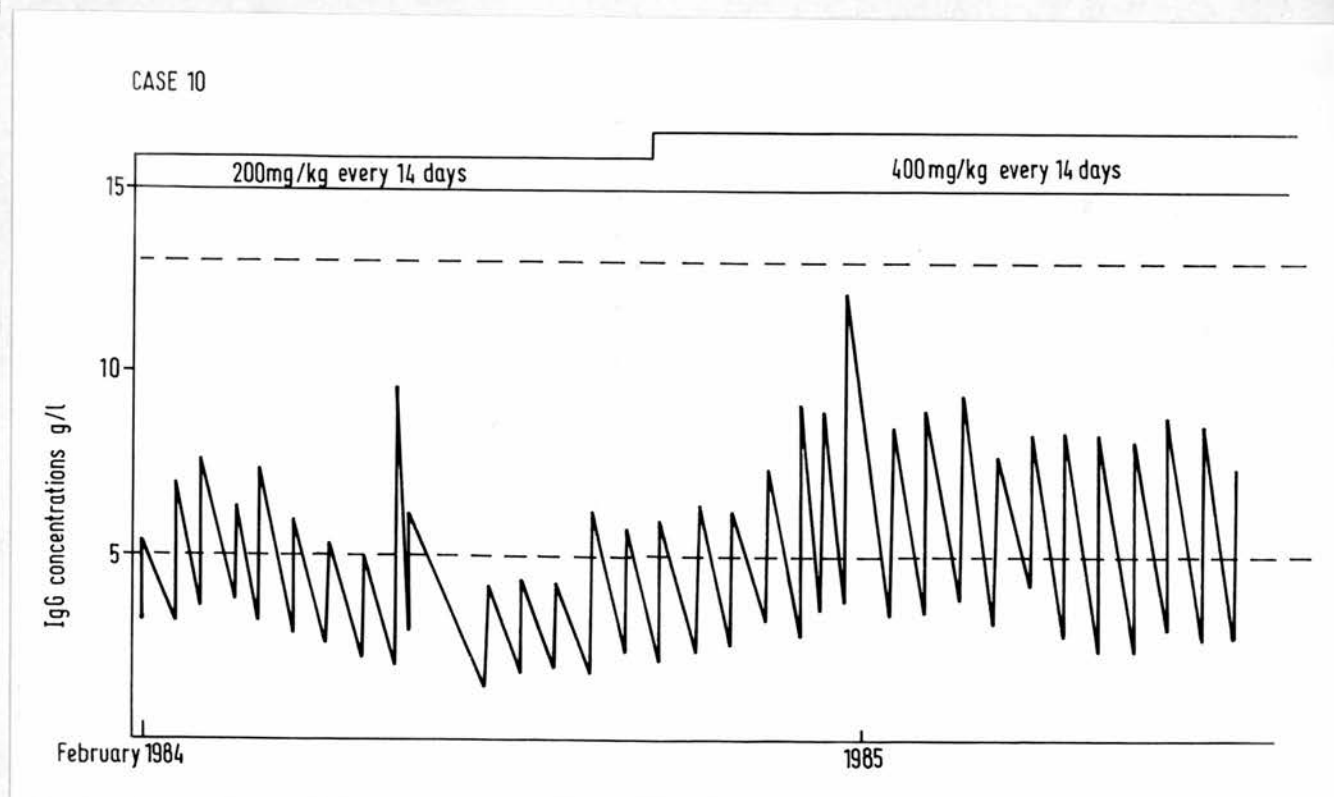


Figure 5.5.4

CASE 11

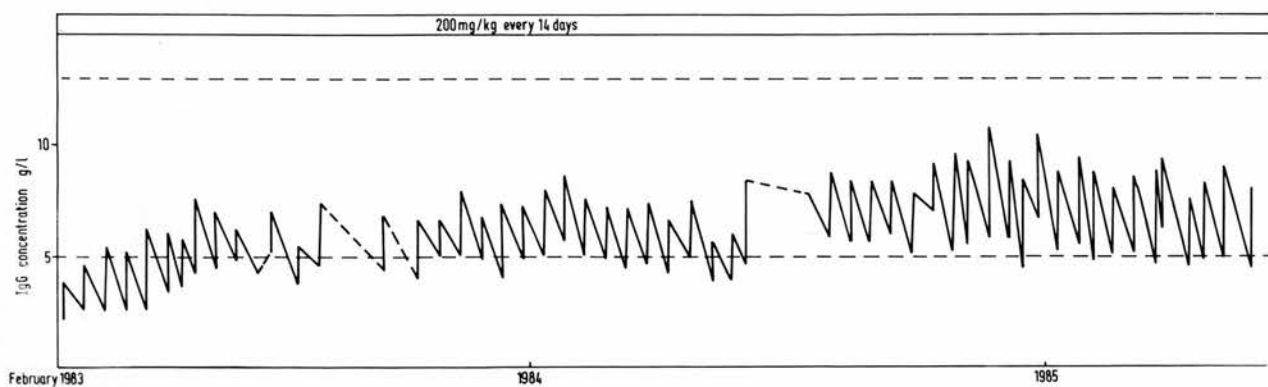


Figure 5.5.5

CASE 12

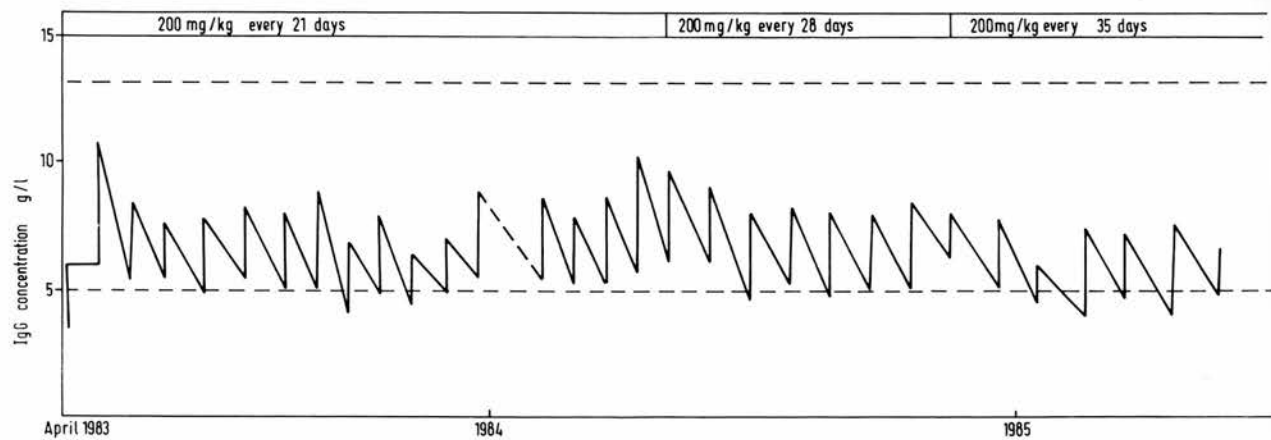


Figure 5.5.6

increasing the infusion frequency (see below).

#### 5.5.4.1.3 Effect of increasing frequency of infusions

In three patients, IgG replacement therapy was carried out once every two weeks because of their low serum IgG despite previous replacement therapy with other intravenous immunoglobulin preparations (Cases 9 and 11), and with FFP (Case 10). In one further patient (Case 6), the frequency of infusion was gradually increased to once weekly when he suffered from recurrent gastrointestinal infections.

The serum IgG level in Case 11 increased from 2.2 g/l to greater than 4.0 g/l after three months (Figure 5.5.5). The serum IgG level in Case 10 who was receiving 800 ml of FFP every three weeks decreased from 3.2 g/l to around 2.0 g/l (Figure 5.5.5) while on an IV IgG replacement regime of 200 mg/kg every two weeks, presumably because of an actual decrease in total IgG infused via the latter replacement regime. However, his serum IgG level gradually improved when his infusion dose was doubled. There was no significant increase in 'trough' serum IgG level noted in Case 9 (Figure 5.5.3). However, when his clinical state worsened attempts at further increasing his serum IgG levels were made by weekly infusions of IgG (see below).

In one patient (Case 6), the serum IgG level reflected the severity of his gastrointestinal disease

(Figure 5.5.2). His serum IgG initially fell when he had diarrhoea. Increasing the frequency of SNBTS IV IgG from three-weekly to two-weekly and then to weekly intervals brought his trough serum IgG level in the normal range. However, with a worsening of his gastrointestinal symptoms, his serum IgG level fell again despite the increased frequency of the immunoglobulin administration. Following resection of the strictured and ulcerated segment of ileum found at laparotomy, his serum IgG levels were once again in the normal range while receiving replacement therapy at three-weekly intervals. Histological examination of the resected bowel showed ulceration and lymphoid hyperplasia without any evidence of lymphoma or of Crohn's disease.

#### 5.5.4.1.4 Effect of increasing infusion dose and infusion frequency

One patient (Case 9) with X-linked primary hypogammaglobulinaemia had his infusion dose as well as his infusion frequency increased when his clinical condition deteriorated. He had persistently low levels despite an increased dose of SNBTS IV IgG (300 mg/kg) and an increase in frequency of treatment to fortnightly intervals. He has a combination of structural lung damage and gastrointestinal disease as a result of recurrent infections. The infusion dose was increased to 540 mg/kg every two weeks without any significant

increase in trough serum IgG concentration. However, at a later date while an in-patient in hospital because of abdominal pain, he received 540 mg/kg at weekly intervals and his 'trough' serum IgG level rose to 4.05 g/l (Figure 5.5.3).

#### 5.5.4.1.5 Effect of reducing infusion frequency

In five patients (Cases 1,3,7,12 and 17) the 'trough' serum IgG level was maintained above 4.0 g/l when the frequency of infusion was increased to once every four weeks. With an infusion frequency of once every four weeks Cases 1, 3 and 12 maintained their 'trough' serum IgG level above 5.0 g/l. Figure 5.5.6 illustrates the effect of maintenance of serum IgG level with reduction in the frequency of infusion.

In four patients (Cases 1, 3, 7 and 12) a 'trough' serum IgG level of greater than 4.0 g/l was still achieved when the frequency of infusions was further reduced to once every five weeks.

One patient (Case 3) maintained a high 'trough' serum IgG level of 8.49 g/l 16 weeks after her last infusion of SNBTS IV IgG of 200 mg/kg, the last but one infusion having been carried out 12 weeks previously (Figure 5.5.1).

#### 5.5.4.2 Clinical Efficacy

The clinical outcome of the 17 patients in this



study is documented in Table 5.5.1. Of the 13 patients with serum IgG levels above 4 g/l, all but one (Case 5) experienced a reduction in the number of respiratory infections; Case 5 had long-standing severe bilateral bronchiectasis. Case 16 with bronchiectasis continued to suffer from respiratory infections when his serum IgG level fell from 6.0 g/l while receiving treatment with FFP to 2.7 g/l following treatment with IV IgG.

The frequency of diarrhoea in three further patients (Cases 2, 6 and 13) was unchanged when their serum IgG increased to above 4 g/l. Cases 9 and 10 suffered from chronic diarrhoea despite serum IgG levels of 3.05 g/l and 2.8 g/l respectively. Finally, Case 17 received total parenteral nutrition because of his malabsorption and was therefore free from diarrhoea.

Of three patients who developed salmonella gastroenteritis, the one patient who was receiving adequate immunoglobulin replacement did not suffer from invasive salmonellosis. Animal studies have demonstrated a useful role for humoral immunity in salmonellosis and it is therefore possible that adequate replacement therapy with IV IgG in that patient prevented the development of invasive salmonella infection (Leen et al, 1986b).

#### 5.5.5 Discussion

Immunoglobulin replacement therapy for patients

with primary hypogammaglobulinaemia has mainly been in the form of intramuscular injections. In the current recommended doses of immunoglobulin used (25-50 mg/kg/week), a proportion of such patients still suffer from recurrent infections and develop structural damage over a period of years (Bjorkander et al, 1984). The objective of this study was to examine whether it was possible to maintain serum IgG concentrations at a normal or near-normal level over a long period of time using an intravenous immunoglobulin preparation.

Patients with primary hypogammaglobulinaemia usually have serum IgG levels of 2 g/l or less before treatment. With IM IgG therapy at 25 mg/kg/week, the response is variable but serum IgG levels are only raised by 1-2 g/l with the occasional patient showing no increment in IgG level at all. The serum IgG levels achieved in individual patients are variable and argue strongly against the practice of administering the same dose of IgG to all immunodeficient patients (Buckley, 1980). The half-life of infused IgG is very variable among such patients and clinical observations have suggested that it is shorter during intercurrent infection (Stiehm et al, 1966). There is therefore a need to individualise the dosage of infused IgG in patients with hypogammaglobulinaemia.

Attempts to individualise treatment dosage should achieve a high enough 'trough' serum IgG level to

minimise the incidence of infection, using the minimal quantity of IgG required due to cost and with a minimum number of infusions to enable the patient to lead as normal a life as is possible. However, there are conflicting data on the optimal serum IgG level that such patients need to maintain in order to minimise the risk of infection. Janeway et al (1966) have reported that serum IgG levels of about 2 g/l markedly reduced the incidence of serious infections. In contrast, Joller and his colleagues (1980) have concluded that a minimal serum IgG concentration of about 3 g/l in patients with hypogammaglobulinaemia is associated with an acceptably low risk of acute infections. As no definitive clinical trials have been set up to determine the optimum IgG level for protection against infection and other complications of hypogammaglobulinaemia, most clinicians aim to achieve a near-normal serum IgG level in their patients since it is likely that the nearer to normal the serum IgG level, the lower is the incidence of infection.

Previous studies have attempted to individualise the dose of immunoglobulin to achieve a satisfactory serum IgG concentration, but only one study increased the frequency of IgG infusions. Morell et al (1982) demonstrated that the administration of 300 mg/kg/month to patients with antibody deficiency could increase and maintain their serum IgG level between 3.0 and 5.0 g/l.

Higher serum levels can be achieved by disproportionately larger doses of IgG administered at short intervals and sustained therapeutic IgG levels can be obtained with 500 mg/kg of IV IgG (Montanaro and Pirofsky, 1984). However, while most studies have shown satisfactory increments in serum IgG levels following administration of 300mg/kg/month, there are still patients who have persistently low serum IgG levels (Cunningham-Rundles et al, 1984). In one study, Schiff et al (1984 ; 1985) used the half-life of infused IgG to determine the dose required to maintain 'trough' IgG levels above 2.0 g/l in his patients with humoral immunodeficiency. There was no reduction in the number of infective episodes when the patients received the higher dose of IgG but this was probably because the 'trough' serum IgG level achieved was only around 2.0 g/l.

In this study, the serum IgG levels achieved with SNBTS IV IgG therapy at a dose of 200 mg/kg two- of three-weekly were excellent with 'trough' levels in the normal range (greater than 5 g/l) for over a year in 50% of the patients. Moreover, by increasing the infusion dose, or by reducing the interval between two infusions, or by a combination of the two, substantial increments in the 'trough' IgG level in the patients were successfully achieved and maintained.

Sequential serum IgG levels in Case 6 demonstrate

the increased loss of infused IgG in the gastrointestinal tract. In such patients adequate levels can be maintained in the near-normal range with frequent infusions of IgG with the usual dose of 200 mg/kg. Similar increases could also be achieved in patients by using very high doses - the equivalent of eight times the usual dose of IV IgG, as in Case 9.

When the intervals between infusions were increased from three to five weeks in six selected patients the 'trough' serum IgG level was maintained above 4.0 g/l. One possible explanation for this is that once the total IgG body pool is saturated, the requirement for IgG becomes less and normal trough serum IgG levels are easily maintained. This is consistent with the finding by Lever et al (1987) that the half-life of infused IV IgG is prolonged after nine months of replacement therapy. One patient (Case 3) maintained her serum IgG level in the normal range for 16 weeks after her last infusion. Although she had primary panhypogammaglobulinaemia with a history of recurrent bacterial infections at the time of diagnosis, preliminary tests suggest that she is now suffering from IgG 2-IgA deficiency.

In summary, 76% of the patients achieved a 'trough' serum IgG level of at least 4.0 g/l within eight months of the start of SNBTS IV IgG and 80% of patients maintained their trough serum IgG level above 4.0 g/l

when they received SNBTS IV IgG in a dose of 200 mg/kg every four to five weeks. In patients with large immunoglobulin losses, normal 'trough' serum IgG levels can be achieved by use of larger doses of SNBTS IV IgG by increasing the infusion doses or the frequency of infusions, or by increasing both the frequency and dose of infusions. While most of the patients who achieved high 'trough' serum IgG level, including those with mild to moderate bronchiectasis, improved during the study, the frequency of diarrhoea in those with gastrointestinal disease was unaffected, and one patient with long-standing severe bilateral bronchiectasis continued to suffer from recurrent pyogenic respiratory infections.

## 5.6 CONCLUSION

SNBTS IV IgG was well tolerated by patients with primary hypogammaglobulinaemia; the incidence of adverse reactions was extremely low in this group of patients known to be more likely to suffer from adverse reactions during immunoglobulin replacement therapy. The higher incidence of adverse reactions encountered during the first few IV IgG infusions was reduced by slowing the infusion rate and by the additional use of prophylactic aspirin or other inhibitor of prostaglandin synthesis. Subsequent increase in the dose and rate of infusion did not result in a higher incidence of adverse reactions.

The administration of large amounts of SNBTS IV IgG was not associated with any episodes of hepatitis, including NANB hepatitis, in the patients studied. This suggests that the plasma pool may not have been significantly contaminated with NANB hepatitis virus(es). But it is more likely that during the manufacturing process (mild pepsin proteolysis at pH 4), residual NANB hepatitis virus(es) present in Cohn II fraction were inactivated, suggesting that other viruses may have been likewise inactivated.

The half-life of SNBTS IV IgG was prolonged in patients with primary hypogammaglobulinaemia. This is comparable with other IV IgG preparations containing intact IgG molecules. Individualising the dose and

frequency of IV IgG infusions was successful in correcting low serum IgG levels in most patients. While most patients benefitted from an increased serum IgG level, the frequency of diarrhoea was unaffected and patients with severe structural lung damage may continue to suffer from repeated respiratory infections. Further controlled clinical studies are needed to examine the optimal serum IgG level consistent with freedom from recurrent infection and irreversible structural organ damage.



6 GENERAL DISCUSSION

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## 6.1 MECHANISM OF HUMORAL IMMUNITY

The antibody response resulting from exposure to antigenic substances has certain well-defined characteristics.

Following exposure to antigen, there is a delay of about two weeks before any antibody can be found in the blood and when there is intense activity in the lymphoid tissues (spleen, lymph nodes etc), and in the submucosa of the respiratory and intestinal tracts. This antibody produced during the 'primary immune response' does not reach a high level and does not persist unless a second dose of antigen is given. When this happens, any remaining antibody is rapidly mopped up by combination with the antigen reflected by an initial fall in circulating antibody. Then, after only a day or two a remarkable rise in antibody level begins and within a few days reaches a peak which can be from 10 to 50 times higher than the primary response. This 'secondary response' is maintained at a high level, falling slowly over a period of months. The response can be further boosted to even higher levels by additional injections of antigen (booster or recall dose) until a stage is reached when no further increase occurs.

Protective antibodies generally combine with antigenic components on the surface of microorganisms and prevent them from multiplying, and sometimes kill them. The antimicrobial actions of antibodies can be

categorised as follows:

- 1 Promoting phagocytosis and subsequent digestion of microorganisms by acting as cytophilic antibodies or opsonins
- 2 Preventing attachment of microorganisms to susceptible cells by combining with the surface of microorganisms
- 3 Neutralisation of microbial toxins
- 4 Activating the complement system following their attachment to the microbes, leading to the release of vasoactive substances, and of lysosomal enzymes, leucocyte chemotaxis, immune adherence (opsonisation) and membrane lysis.
- 5 Enhancing phagocytosis following agglutination of small microorganism, and rendering them nonmotile

## 6.2 SUCCESSFUL IMMUNOGLOBULIN THERAPY

For immunoglobulin treatment to be effective, the mechanisms by which antibodies protect against microorganisms should be taken into consideration when immunoglobulin is manufactured.

By the time they reach adult life all animals, including man, have been exposed to a wide variety of infectious agents and have produced antibodies to most of them. Serum immunoglobulin levels reflect this extensive and universal natural process of immunisation. Therefore, pooled plasma from a large number of blood

donors would contain a large repertoire of antibodies against a variety of different microorganisms.

### 6.3 HYPERIMMUNE PLASMA PROCUREMENT

While this pooled plasma can be used for the manufacture of immunoglobulin for replacement in patients with primary humoral immunodeficiency, it would not usually contain high enough levels of specific antibodies to make it suitable for the manufacture of specific hyperimmune globulins. For this purpose, therefore, donors with high levels of specific antibodies have first to be identified before they are enrolled in a plasmapheresis programme.

Such donors usually acquire high levels of a specific antibody through prior natural exposure to the infectious agent, leading to a subclinical or clinical infection, or because they have been immunised against that agent to prevent the infection.

Ways of recruiting donors for the procurement of hyperimmune plasma for the manufacture of specific hyperimmune globulin have been examined in this thesis. There is undoubtedly a need for such immunoglobulin preparations in the prophylaxis and treatment of certain infections (see section 3.3). In order to meet this clinical need, new methods of identifying suitable plasma donors for regular plasmapheresis have to be explored.

The clinical use of immunoglobulins has been reviewed in section 3.3. Undoubtedly, when new organisms are discovered or old organisms assume increasing clinical importance in immunocompromised patients, specific immunoglobulin preparations are likely to be useful until other specific therapeutic agents are discovered, or until safe and effective vaccines become available. An example is CMV, which can cause life-threatening infection in the immunocompromised patient. CMV hyperimmune globulin can certainly modify, if not prevent, CMV infection but since the advent of new antiviral agents its role in the treatment of severe CMV infection is under review. Nonetheless, even when safe and effective vaccines become available, as in the case of rabies, the demand for hyperimmune rabies immunoglobulin in the post-exposure prophylaxis of rabies will remain.

How can the clinical demand for these preparations be met? Adequate numbers of suitable donors with high levels of specific antibodies have to be identified. Identifying these donors has depended on large-scale screening of the blood donor populations which is expensive and time- and labour-intensive. Random screening of the blood donor population is routinely carried out for the identification and recruitment of hepatitis B and CMV plasma donors (sections 4.2 and 4.3). The two studies examining the CMV and hepatitis B

plasma procurement have confirmed the difficulties with such screening programmes, which have to be repeated at frequent intervals to maintain a panel of suitable donors. Many donors are withdrawn from the panel because their antibody levels fall below the minimum required for hyperimmune donations, or because of underlying medical conditions, or because they have moved away from the area.

The suitability of boosting selected donors as a means of recruiting plasma donors has been demonstrated by the tetanus study (section 4.4). With the availability of a suitable and safe vaccine, selected donors can be rendered hyperimmune and their plasma harvested for the manufacture of hyperimmune globulin. The advantages include planned programmes of hyperimmunisation and subsequent plasma collection to meet clinical demands which could be repeated at regular intervals in a controlled way, ensuring ease of adequate plasma availability. This is certainly possible with tetanus toxoid, and could well be feasible for other hyperimmune plasma collection (Table 4.1).

#### 6.4 EVALUATION OF pH 4/PEPSIN TREATED IV IgG PREPARATION

SNBTS IV IgG, a new intravenous immunoglobulin preparation manufactured by the pH4/ mild pepsin method, was been evaluated in patients with primary hypogammaglobulinaemia (sections 5.2, 5.3, 5.4 and

5.5).

Although these patients are more prone to experience adverse reactions following the administration of IgG, the incidence of such reactions to the new preparation was low and mostly mild, with the majority of reactions occurring with the initial infusions. Reactions could still occur many months after the patient had been established on regular IV IgG. Only four adverse reactions were moderately severe, and no patient was intolerant of the product. The incidence of adverse reactions was further reduced by slowing the infusion rate of the IV IgG administered in reduced amounts to patients who had never previously received any form of IgG. The prophylactic use of aspirin was thought to be of benefit to one patient who experienced fever and influenza-like symptoms following IV IgG. Finally, although much is known about the cause of adverse reactions, the pathogenesis of most adverse reactions remains unclear in many patients. In this study (section 5.2), for example, there was no evidence of complement activation and no definite cause for the reactions was ever found, but the general impression was that the actual rate of infusion might have been an important factor. Infusion rates of 264 mg/kg/hr were tolerated without adverse reactions once the patients were well established on IV IgG therapy.

The half-life of SNBTS IV IgG in some patients with



hypogammaglobulinaemia was longer than four weeks (range 15-53 days) (section 5.4). This was comparable with other IV IgG preparations that are currently available for clinical use. Schiff (1985) reported that in patients with primary hypogammaglobulinaemia the half-life of infused IgG was not dependent on the serum IgG level. However, Lever et al (1987) have found an increase in the half-life of IgG at the end of a nine-month course of SNBTS IV IgG treatment. This may be due to a general decrease in protein catabolism associated with an improvement in the patients' clinical status accompanying an increase in serum IgG level.

The wide variation in the half-life of infused IgG would explain the difficulty in achieving high normal serum IgG in such patients when using standard IgG doses of 200 mg/kg every four weeks. This can be overcome by tailoring the dose and frequency of infusion of IV IgG to suit the particular needs of the patient (section 5.5). Indeed, 13 of 17 patients studied achieved a serum 'trough' IgG level of greater than 4 g/l. While most patients studied benefitted from the intravenous administration of IgG, two patients with severe bilateral bronchiectasis whose serum IgG was previously maintained in the normal range while receiving FFP did not notice any improvement in their chronic respiratory symptoms.

Studies examining the efficacy of IgG in patients

with primary hypogammaglobulinaemia are very difficult to set up. Most of the published trials have compared the incidence of infection while the patients were receiving either intramuscular or intravenous immunoglobulin, and they have shown the IV IgG to be more beneficial to the patients. However, few clinical trials have taken into consideration variations in the seasonal incidence of infection. Trials, therefore, should run for a prolonged period of time, preferably at least two years, and have a cross-over design to avoid seasonal variations in infection rate. The trials should also compare the infection rate in patients with different serum IgG levels. This would then allow physicians to decide upon the optimal serum IgG level required to minimise the frequency of infections and to prevent structural lung damage. Finally, these studies require the total cooperation of patients for the recordings of antibiotic usage and episodes of fever and days off work to be accurate. With the prevalence of primary hypogammaglobulinaemia being so low, such studies are, therefore, extremely difficult to set up and smaller studies are probably unsuitable.

Cohn fraction II has an excellent record in terms of safety from hepatitis B transmission, and the few outbreaks of hepatitis associated with the immunoglobulin are thought to be due to some fault in the processing of a particular batch rather than to an

inherent unreliability of the fractionation process. This was reinforced by a study by Murray and Ratner (1953), who found that IgG prepared by cold ethanol fractionation, from a starting plasma that was icterogenic and highly infectious, was not infectious to volunteers. The starting plasma was later shown to contain hepatitis B virus. Later studies of plasma containing hepatitis B surface antigen with all the markers of infectivity showed that, after cold ethanol fractionation, the resultant IgG was free of all these markers which were found in fraction III (Trepo et al, 1978). Furthermore, although hepatitis B surface antigen was rarely found in fraction II, studies using ultracentrifugation revealed small amounts of hepatitis B surface antigen complexed with hepatitis B surface antibody in fraction II and these complexes were not infectious (Hoofnagle and Waggoner, 1980). Finally, as all blood donations are now screened for hepatitis B surface antigen, immunoglobulin is unlikely to contain these non-infectious complexes.

NANB hepatitis has been transmitted by several plasma fractions (Kernoff et al, 1984 ; Fletcher et al, 1983), but until 1981 there had been no evidence of transmission of NANB by any of the immunoglobulin preparations. An outbreak of NANB hepatitis after the administration of Rh(D) immunoglobulins was reported by Renger et al (1981). But it was the three outbreaks of

NANB hepatitis in patients with primary hypogammaglobulinaemia receiving IV IgG preparations in the United Kingdom, United States and Finland that have challenged the view that cold ethanol fractionation inactivates NANB hepatitis viruses regardless of the infectivity titre present in the starting pool. While SNBTS IV IgG and other pH4/mild pepsin treated IV IgG (e.g. Sandoglobulin and SNBTS IV IgG) have so far been free of NANB hepatitis transmission (section 5.3), it could be argued that this is because the original plasma pool may well have been free of NANB virus(es). However, this is unlikely to be the case, and indeed, it is thought that the manufacture of the IV IgG preparations implicated in the outbreaks of NANB hepatitis may not have included a known virucidal step and therefore yielded an infective product when the initial plasma pool was contaminated by a viraemic donor.

The outbreaks of NANB hepatitis are also related to the use of large doses of IV IgG. Since the dose of IV IgG used is 50 to 100 times higher, the infectivity level may be more easily reached than with standard intramuscular IgG.

At the present time, a suitable marker for NANB hepatitis is not available and while chimpanzee inoculation can be used to test for NANB hepatitis transmission, this is expensive and time-consuming and unreliable unless properly conducted (Prince, 1985).

Furthermore, there are only a limited number of chimpanzees available throughout the world. Until more reliable tests become available screening all plasma donations for abnormally high ALT levels would decrease the risk of NANB transmission (Hollinger, 1984). However, this should not obviate the need for regular and meticulous screening of the recipients for evidence of hepatitis infection. Hepatitis infectivity is usually indicated by elevated ALT or AST levels (two times the upper limit of normal) over two consecutive monthly samples, in the absence of other known causes of elevated liver enzymes.

#### 6.5 AIDS AND PLASMA DERIVED PRODUCTS

Today attention has shifted away from hepatitis transmission to the question of the transmissibility of AIDS through plasma derived products. The risk of gammaglobulin transmitting AIDS has generated some concern. This is because gammaglobulin is a pooled product from many donors which is not pasteurised. However, although gammaglobulins are widely used there has been only one reported case of a person not in a high-risk group who has received immunoglobulin prior to the onset of AIDS (MMWR, 1983). This patient received immunoglobulin prophylactically for a needle stick puncture from an unknown source. Furthermore, while HIV antibody has been detected in lots of gammaglobulin and

hepatitis B immune globulin, these lots have all been culture negative for the virus (Tedder et al, 1985; Gocke et al, 1986; Ikeda et al, 1986).

Intravenous gammaglobulin prepared by the reduction and alkylation method, or pH 4/pepsin treatment method, was found to contain antibodies against HIV using ELISA and Western blot analysis. The gammaglobulin preparations showed no signs of viral activity (by monitoring for reverse transcriptase activity or virus antigen expression). Recipients of large doses of the preparations became transiently seropositive but the antibody was transient and was not associated with infection (Wood et al, 1986).

The isolation of retroviruses related to HIV from two patients with 'common variable' hypogammaglobulinaemia by Webster and his colleagues (1986) raised the possibility of these viruses being transmitted by contaminated IV IgG. However, since there have been no other reports of clinical AIDS in patients receiving IV IgG, they raised the possibility that these retroviruses may be the cause of 'common variable' hypogammaglobulinaemia. But there has been difficulty in isolating similar viruses in other patients with 'common variable' hypogammaglobulinaemia (Webster, personal communication 1986).

In view of the widespread and frequent use of gammaglobulin and other Cohn fraction II derivatives

such as hepatitis B immune globulin, and the lack of clinical AIDS being manifest in their recipients, one cannot consider it to be a significant risk for transmitting this disease. The most likely explanation of its safety relates to the cold ethanol preparation used in Cohn fractionation being able to denature the virus (Spire et al, 1984; Martin et al, 1985; Piszkiwicz et al, 1985). It has been reported that 25% ethanol inactivates HIV virus. In addition, the virus probably precipitates during fractionation procedures into fraction I and the cold insoluble fraction, thereby sparing Cohn fraction II (Wells et al, 1986).

While it is now generally accepted that all plasma pools used in the manufacture of blood products are likely to contain viral contamination, attempts should be made to limit the contamination to a minimum in order to minimise the demands on any inactivation processes during the manufacture of blood products. The most efficient means of excluding infective donations is by the use of assays for specific markers of potential infectivity, e.g. hepatitis B antigen, HIV antibody, hepatic enzymes. The exclusion of some infective donations can also be achieved by persuading donors in 'high risk' groups, particularly homosexuals and intravenous drug abusers, to abstain from donating blood or plasma.

During the manufacture of IV IgG, further processing

of the IgG is required following the removal of ethanol to reduce the level of spontaneous anti-complementary activity and of vasoactive enzymes. Several of the techniques used at this stage have recognised or potential virucidal activity. These include beta-propiolactone and ultraviolet irradiation (Prince et al, 1983), acid treatment (Andrewes et al, 1978) and limited or complete proteolysis using pepsin at low pH (Tabor et al, 1983).

## 6.6 FINAL CONCLUSION

In this thesis it has been shown that random screening of donors for high levels of specific antibody is not effective, being time- and labour-intensive and costly. This has been confirmed for hepatitis B and CMV plasma procurement. The study on boosting donors with tetanus toxoid for the procurement of tetanus plasma has shown that donors can be hyperimmunised without an excess of adverse reactions. This study has also identified the donors most suitable for boosting with tetanus toxoid prior to plasmapheresis. While similar studies could be set up to examine the feasibility of boosting donors with other vaccines for hyperimmune plasma procurement, the availability of safe and effective vaccines remains the crucial factor. Indeed, while donors can be boosted with hepatitis B vaccine, there is not a completely safe and effective CMV



vaccine. The demonstration of an effective and safe boosting policy with tetanus toxoid would therefore pave the way to hyperimmunisation of donors for the procurement of other specific hyperimmune plasma.

The studies on adverse reactions and risk of hepatitis transmission have confirmed the safety of a pH 4/mild pepsin treatment IV IgG product. Patients with primary hypogammaglobulinaemia who have never previously received any immunoglobulin replacement are now given a small initial dose of IV IgG with or without any prophylactic aspirin. Following the study on dosage individualisation of IV IgG for each patient with hypogammaglobulinaemia, the dose and frequency of administration of IV IgG is now regularly monitored so as to maintain the 'trough' serum IgG level as near the normal range as possible. This may be important as it is likely that if normal serum IgG levels can be achieved as soon as the diagnosis is made, permanent structural organ damage is likely to be minimal.

There is no doubt that further, properly-conducted studies in to whether patients with primary hypogammaglobulinaemia benefit from achieving normal serum 'trough' IgG level are required, and it is only when these studies are completed that we will know if the treatment of these patients is more complex than merely correcting the deficiency of antibodies.

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APPENDIX A  
PATIENTS STUDIED

## PATIENT 1

Date of Birth : 15.01.51  
Age at diagnosis : 34 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Painful lymphadenopathy  
Recurrent infection  
Past medical history: Autoimmune haemolytic anaemia  
Eczema

Family history : nil of note

Duration of study : 6 months

	Before study	After study
Serum IgG	: 3.35 g/l	5.52 g/l

Details and progress: well since on IV IgG.

## PATIENT 2

Date of Birth : 10.09.52  
Age at diagnosis : 26 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Weight loss and diarrhoea  
Intractable giardiasis  
Past medical history: Insulin dependent diabetes mellitus  
Sarcoidosis

Family history : nil of note

Duration of study : 32 months

	Before study	After study
Serum IgG	: 5.47 g/l	6.75 g/l

Details and progress: Prestudy serum IgG is not low because of prior treatment with other IV IgG preparations. Well since on IV IgG. He is the subject of a case report (Leen et al, 1985b).

### PATIENT 3

Date of Birth : 03.04.71  
Age at diagnosis : 11 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Recurrent infections  
Past medical history: Asthma  
Idiopathic thrombocytopenia  
Herpes Zoster aged 7 and 12 years  
Multiple allergies

Family history : mother has Hashimoto's thyroiditis

Duration of study : 9 months

	Before study	After study
Serum IgG	: 1.81 g/l	5.21 g/l

Details and progress: well since on IV IgG.

### PATIENT 4

Date of Birth : 09.01.63  
Age at diagnosis : 14 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Recurrent infections  
Past medical history: Recurrent herpes labialis  
Nodular lymphoid hyperplasia  
Chronic diarrhoea

Family history : nil of note

Duration of study : 22 months

	Before study	After study
Serum IgG	: 2.5 g/l	6.15 g/l

Details and progress: well since on IV IgG, and diarrhoea improved.

#### PATIENT 5

Date of Birth : 13.09.58  
Age at diagnosis : 7 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Recurrent infections  
Past medical history: Recurrent sinusitis  
Meningococcal meningitis  
Herpes Zoster  
Giardiasis

Family history : nil of note

Duration of study : 33 months

	Before study	After study
Serum IgG	: 4.34 g/l	10.4 g/l

Details and progress: well since receiving immunoglobulin replacement. Prestudy serum IgG is not low because of previous IV IgG therapy. Three years after regular IV IgG, she now has normal serum IgG levels but probably has IgA deficiency with IgG 2 subclass deficiency. She is well without IgG replacement.

#### PATIENT 6

Date of Birth : 22.03.62  
Age at diagnosis : 7 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Severe lobar pneumonia  
Past medical history: Recurrent sinusitis  
Lobar pneumonia X 3

Family history : nil of note

Duration of study : 13 months

	Before study	After study
Serum IgG	: 1.53 g/l	4.16 g/l

Details and progress: well since receiving immunoglobulin replacement. He experienced adverse reactions during the initial IV IgG infusions but settled after prophylactic aspirin.

## PATIENT 7

Date of Birth : 15.02.55  
Age at diagnosis : 24 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Recurrent chest infections  
Past medical history: Recurrent sinusitis  
Herpes labialis  
Bilateral bronchiectasis  
Cachexia due to severe lung sepsis  
Digital warts

Family history : nil of note

Duration of study : 14 months

	Before study	After study
Serum IgG	: 8.08 g/l	5.6 g/l

Details and progress : no obvious improvement despite reasonable serum IgG levels; bronchiectasis remains a problem and because of severe sepsis she is underweight.

## PATIENT 8

Date of Birth : 03.04.59  
Age at diagnosis : 19 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Recurrent upper respiratory infections  
Past medical history: Insulin dependent diabetes mellitus  
Recurrent Otitis Media  
Hepatitis  
Flexural eczema, alopecia  
Steatorrhoea  
IgA antibodies  
Multiple allergies e.g. insulin  
Salmonellosis  
Perirectal abscess

Family history : nil of note

Duration of study : 10 weeks

	Before study	After study
Serum IgG	: 2.35 g/l	4.76 g/l

Details and progress: clinically free from severe infection but recurrent sinus and ear infections.

## PATIENT 9

Date of Birth : 19.03.42  
Age at diagnosis : 30 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : severe staphylococcal pneumonia  
Past medical history: Pulmonary tuberculosis  
Infective hepatitis  
Coeliac disease  
Giardiasis  
Campylobacter enteritis  
Small bowel strictures, ? crohns

Family history : nil of note

Duration of study : 32 months

	Before study	After study
Serum IgG	: 2.73 g/l	4.82 g/l

Details and progress: clinically free from severe infection and serum IgG better maintained after resection of diseased segment of bowel.

## PATIENT 10

Date of Birth : 06.04.21  
Age at diagnosis : 54 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Weight loss and diarrhoea  
Past medical history: Pulmonary tuberculosis  
Duodenal ulcer  
Vagotomy & Pyloroplasty  
Seropositive Rheumatoid Arthritis  
Manic depression

Family history : nil of note

Duration of study : 5 months

	Before study	After study
Serum IgG	: 2.68 g/l	5.25 g/l

Details and progress: clinically free from severe infection. She became rheumatoid factor negative when diagnosed as suffering from hypogammaglobulinaemia.

#### PATIENT 11

Date of Birth : 22.11.54  
Age at diagnosis : 27 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Severe pneumococcal pneumonia  
Past medical history: Recurrent Otitis Media  
Myringoplasty

Family history : nil of note

Duration of study : 33 months

	Before study	After study
Serum IgG	: 6.24 g/l	4.5 g/l

Details and progress: clinically free from severe infection. His high serum IgG before the study is the result of IgG replacement therapy with fresh frozen plasma and at least two other IV IgG preparations.

#### PATIENT 12

Date of Birth : 22.10.29  
Age at diagnosis : 46 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Recurrent pneumonia  
Past medical history: Sinusitis  
Pernicious anaemia  
Giardiasis  
Purulent conjunctivitis  
Severe reaction to IMIgG  
Appendicitis  
Mouth ulcers  
Arthritis  
Renal biopsy proven Amyloidosis

Family history : nil of note

Duration of study : 8 months

	Before study	After study
Serum IgG	: 1.40 g/l	3.09g/l

Details and progress : clinically free from severe infection. The severe proteinuria secondary to his amyloid appears to have spontaneously resolved following treatment with IM IgG.



### PATIENT 13

Date of Birth : 01.10.47  
Age at diagnosis : 24 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms : Recurrent chest infections  
Past medical history: Recurrent Sinusitis  
Otitis Media  
Hepatitis A  
Sarcoidosis  
Bronchiectasis  
Severe Herpes Zoster

Family history : nil of note

Duration of study : 15 months

	Before study	After study
Serum IgG	: <0.97 g/l	4.17 g/l

Details and progress ; clinically free from severe infection. Since receiving IV IgG he has gained 12 pounds in weight. Chest X ray findings are, however, unchanged. He is the subject of a case report (Leen et al, 1985b).

# PATIENT 14

Date of Birth : 30.05.64  
Age at diagnosis : 30 months  
Diagnosis : X-linked Hypogammaglobulinaemia  
Presenting symptoms : Recurrent infections  
Past medical history: Shigella Sonnei dysentery  
Bronchiectasis  
Malabsorption without villous atrophy  
Recurrent mucosal ulcers of uncertain  
aetiology  
Jejuno-ileal strictures, ?Crohn's  
Recurrent otitis media and sinusitis

Family history : X-linked hypogammaglobulinaemia

Duration of study : 33 months

	Before study	After study
Serum IgG	: 1.46 g/l	4.48 g/l

Details and progress : In 1980, treatment with fresh frozen plasma started because of low serum IgG levels while on IM IgG. In 1981, investigations for weight loss showed no treatable abnormality. In 1986, investigations for abdominal pain, weight loss, and severe iron deficiency anaemia revealed ulceration, tubular narrowing, saculation and thickening of bowel wall together with effacement of mucosal folds and a cobblestone appearance most marked in the proximal bowel. Resection of the diseased portion of the bowel resulted in a 7 pound weight gain and normalisation of serum IgG levels over a six month period. He remains well.

## PATIENT 15

Date of Birth : 14.04.67  
Age at diagnosis : 6 months  
Diagnosis : X-linked Hypogammaglobulinaemia  
Presenting symptoms: Screened because brother is known  
to have Hypogammaglobulinaemia  
Past medical history: Diarrhoea due to total villous atrophy  
Hypoparathyroidism  
Family history : X-linked hypogammaglobulinaemia  
Duration of study : 21 months

	Before study	After study
Serum IgG	: 3.22 g/l	2.55 g/l

Details and progress : Treatment with fresh frozen plasma started because of low serum IgG levels while on IM IgG explains reasonable serum IgG before study. Hypoparathyroidism diagnosed in 1985 and he is maintained on 4 mcg of one-alpha every day. He does not have any stigmata of hypoparathyroidism.

## PATIENT 16

Date of Birth : 14.08.72  
Age at diagnosis : 22 months  
Diagnosis : X-linked Hypogammaglobulinaemia  
Presenting symptoms: Recurrent infections  
Past medical history: Otitis Media  
Left lower lobe pneumonia  
Bilateral bronchiectasis  
Left ureteronephrectomy  
Giardiasis  
Congestive cardiac failure  
Hypertension  
Campylobacter enteritis

Family history : nil of note

Duration of study : 33 months

	Before study	After study
Serum IgG	: 2.24 g/l	3.79 g/l

Details and progress: In 1979, investigations of a left paravertebral mass resulted in a laparotomy revealing a renal mass; the histology of which suggested a mycotic aneurysm. Surgery was difficult and a left ureteronephrectomy was performed. Treatment with fresh frozen plasma started in 1980, and subsequently received two IV IgG preparations. He needed increased diuretics during IV IgG infusions because of his cardiac failure. Since last studied in November 1985, he developed a septicæmic illness and died in 1986.

## PATIENT 17

Date of Birth : 06.10.62  
Age at diagnosis : 18 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms: Bronchiectasis  
Past medical history: Infectious Mononucleosis  
Uneventful pregnancy

Family history : nil of note

Duration of study : 32 months

	Before study	After study
Serum IgG	: 3.36 g/l	5.05 g/l

Details and progress : She received two IV IgG preparations prior to the study. She delivered a normal male baby in 1985. The baby's serum IgG was normal because of passively transferred IgG from a well maintained serum IgG in the mother. Baby does not have hypogammaglobulinaemia. She is now well and has had no serious lung sepsis since on IV IgG.

## PATIENT 18

Date of Birth : 16.12.58  
Age at diagnosis : 18 years  
Diagnosis : Common variable hypogammaglobulinaemia  
Presenting symptoms: Recurrent chest infections  
Past medical history: Right upper lobe pneumonia  
Diarrhoea  
Recurrent conjunctivitis

Family history : nil of note

Duration of study : 6 months

	Before study	After study
Serum IgG	: 2.00 g/l	4.98 g/l

Details and progress : Treatment with intravenous immunoglobulin improved his chest but his diarrhoea remains a problem.

## PATIENT 19

Date of Birth : 22.06.47  
Age at diagnosis : 30 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms: Recurrent Chest Infections  
Past medical history: Otitis Media & Sinusitis  
Bell's palsy  
Pernicious anaemia  
Herpes Zoster  
Giardiasis  
Salmonella septicaemia

Family history : nil of note

Duration of study : 15 months

	Before study	After study
Serum IgG	: 1.57 g/l	4.91 g/l

Details and progress: Treatment with IM IgG did not improve her chronic bronchitis. She developed severe salmonella septicaemia while pregnant and lost the baby she was carrying. Since on IV IgG from August 1984, she has been able to stop her prophylactic antibiotic without any deterioration of her chest symptoms.

## PATIENT 20

Date of Birth : 09.08.34  
Age at diagnosis : 49 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms: Recurrent Chest Infections  
Past medical history: Pernicious Anaemia  
Pneumonia X 3 every year

Family history : nil of note

Duration of study : 19 months

	Before study	After study
Serum IgG	: 1.81 g/l	4.39 g/l

Details and progress : Since treatment with IV IgG she has not had any lower respiratory symptoms. She feels much better and more active.

#### PATIENT 21

Date of Birth : 31.10.34  
Age at diagnosis : 29 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms: Recurrent Chest Infections  
Past medical history: Pyogenic Arthritis  
Hypersensitive to IM IgG

Family history : nil of note

Duration of study : 8 months

	Before study	After study
Serum IgG	: 4.23 g/l	5.60 g/l

Details and progress : Since replacement therapy has been changed from IM IgG to fresh frozen plasma, his symptoms have vastly improved. While on IV IgG, he has been able to stop his prophylactic antibiotics and has experienced no deterioration.

#### PATIENT 22

Date of Birth : 16.09.60  
Age at diagnosis : 22 years  
Diagnosis : X-linked Hypogammaglobulinaemia  
Presenting symptoms: Recurrent Chest Infections  
Past medical history: Pneumonia  
Meningitis

Family history : nil of note

Duration of study : 31 months

	Before study	After study
Serum IgG	: 3.92 g/l	4.05 g/l

Details and progress : He had received two IV IgG preparations prior to the start of the study. He is known to have a large intake of alcohol and this has explained his occasional high serum ALT levels. He remains free of serious infective problems.

### PATIENT 23

Date of Birth : 30.11.63  
Age at diagnosis : 13 years  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms: Recurrent Chest Infections  
Past medical history: Febrile convulsions  
Temporal Lobe Epilepsy  
XXX syndrome  
Salmonella enteritis  
Giardiasis

Family history : nil of note

Duration of study : 9 months

	Before study	After study
Serum IgG	: 2.41 g/l	2.60 g/l

Details and progress : She is free from serious infections, but her main problem is epilepsy.

### PATIENT 24

Date of Birth : 28.01.65  
Age at diagnosis : 30 months  
Diagnosis : X-linked Hypogammaglobulinaemia  
Presenting symptoms: Recurrent Chest Infections  
Past medical history: Severe encephalitis with  
mental retardation, and  
Epilepsy  
Bronchiectasis

Family history : nil of note

Duration of study : 30 months

	Before study	After study
Serum IgG	: 6.06 g/l	3.49 g/l

Details and progress : His replacement therapy has been changed from IM IgG to fresh frozen plasma because his chest symptoms were deteriorating. However, while on IV IgG, they remain unchanged.



## PATIENT 25

Date of Birth : 13.11.42  
Age at diagnosis : late twenty's  
Diagnosis : Common Variable Immunodeficiency  
Presenting symptoms: Chronic diarrhoea  
Past medical history: Malabsorption and weight loss  
Chronic hepatitis  
Cholecystitis  
Giardiasis

Family history : nil of note

Duration of study : 21 months

	Before study	After study
Serum IgG	: 2.86 g/l	4.19 g/l

Details and progress: In view of his severe malabsorption, he was established on home total parenteral nutrition. Investigations carried out in Michigan (USA), revealed that he had defective cellular immunity. He underwent cholecystectomy for cholecystitis and cholelithiasis. A liver biopsy showed portal triaditis with occasional granulomatous foci. Jejunal biopsy showed villous atrophy. He had gastric achlorrhya with absent parietal cell antibodies. In 1984 investigations of abnormal liver function tests including a liver biopsy revealed that they were secondary to an excess lipid in his parenteral nutrition regime. He was killed in a road traffic accident in December 1984.

APPENDIX B  
PUBLICATIONS ARISING

The following papers have been published relating to work described in this thesis :

Leen, C.L.S.; Yap, P.L.; McClelland, D.B.L.; Atrah, I.; and Crawford, R. (1985). Non-A,non-B hepatitis and intravenous immunoglobulin. *Lancet*; i:587-588.

Leen, C.L.S.; Bath, J.C.J.L.; Brettles, R.P.; and Yap, P.L. (1985). Sarcoidosis and primary hypogammaglobulinaemia: A report of two cases and a review of the literature. *Sarcoidosis*; 2 ;91-95.

Leen, C.L.S.; Wilson, R.; Yap, P.L.; and McClelland, D.B.L. (1985). Immune hepatitis B plasma procurement. *Vox Sanguinis*; 49: 323-326.

Leen, C.L.S.; Yap, P.L.; Neill, G.; McClelland, D.B.L.; and Westwood, A. (1986). Serum ALT levels in patients with primary hypogammaglobulinaemia receiving replacement therapy with intravenous immunoglobulin or fresh frozen plasma. *Vox Sanguinis*; 50 : 26-32.

Leen, C.L.S.; Birch, A.D.J.; Brettles, R.P.; and Yap, P.L. (1986). Salmonellosis in patients with primary hypogammaglobulinaemia. *Journal of Infection*; 12 ; 241-245.

Leen, C.L.S.; Barclay, G.R.; and McClelland, D.B.L. (1986). Selection of plasma donors suitable for tetanus boosting. *Vox Sanguinis*; 51: 197-201.

Leen, C.L.S.; Yap, P.L.; and McClelland, D.B.L. (1986). Increase of serum IgG level into the normal range in primary hypogammaglobulinaemia by dosage individualisation of IV IgG. *Vox Sanguinis*; 51: 278-286.

Leen, C.L.S.; Barclay, G.R.; McClelland, D.B.L.; Shepherd, W.T.; and Langford, D.T. (1987). Double blind comparative trial of standard (commercial) and immune purified tetanus toxoids. *Journal of Infection* 14: 119-124.

Leen, C.L.S.; Williams, P.E.; Yap, P.L.; and McClelland, D.B.L. (1987). Tolerance and adverse reactions to intravenous immunoglobulin prepared by mild pepsin proteolysis at pH4 in patients with primary hypogammaglobulinaemia. *Scottish Medical Journal* (in press).

chronic peritoneal dialysis catheter under local anaesthesia. Venous blood was sampled regularly for measurement of plasma morphine concentrations by radioimmunoassay.<sup>1</sup> This method gives identical values to those of high-performance liquid chromatography in patients with renal failure.<sup>2</sup> No further analgesic medication was required since the single dose of morphine had a prolonged clinical effect. Postoperatively the patient appeared sedated for 24 h and was drowsy with constricted pupils for a further 3 days. She had no abnormalities of hepatic function and took no other analgesic medications; she was put on peritoneal dialysis 36 h after insertion of the catheter.

The figure shows plasma morphine concentrations after the single intravenous injection. Morphine was still detectable 19 days after this dose, with an estimated half-life of 73.1 h and a systemic clearance of 23 ml/min. The half-life was much longer and clearance much lower than for patients with normal renal function. Systemic morphine clearance in patients undergoing lumbar laminectomy had a mean of 486 ml/min and range of 189–1051 ml/min.<sup>1</sup>

The remarkable prolongation of the clinical effect of the single 10 mg dose of morphine, paralleled by the long half-life and low systemic clearance, led us to abandon the study. We do not feel that further use of morphine premedication is acceptable in patients with severe chronic renal failure and believe that this patient illustrates the overriding relevance of renal mechanisms for clearance of morphine in these patients.

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1. Moore RA, Baldwin D, Allen MC, et al. Sensitive and specific morphine radioimmunoassay with iodine label: Pharmacokinetics of morphine in man after intravenous administration. *Ann Clin Biochem* 1984; **21**: 318–25.

2. Moore RA, Sear J, Baldwin D, et al. Morphine kinetics during and after renal transplantation. *Clin Pharmacol Ther* 1984; **35**: 641–45.

#### NON-A, NON-B HEPATITIS AND INTRAVENOUS IMMUNOGLOBULIN

SIR,—There have been two reports of non-A, non-B (NANB) hepatitis in patients with hypogammaglobulinaemia given immunoglobulin preparations treated to render them suitable for intravenous use.<sup>1,2</sup> We would like to report on our experience with Scottish National Blood Transfusion Service intravenous immunoglobulin (IVIG) over the past two years. This product is prepared at the Service's Protein Fractionation Centre from a cold ethanol fraction II which is subsequently ultrafiltered, treated by mild pepsin proteolysis at pH 4, and freeze dried.<sup>3,4</sup> The product after reconstitution with water is a solution of 5% (5 g per 100 ml) of human normal immunoglobulin. Patients with primary hypogammaglobulinaemia receive 200–400 mg/kg every 2–5 weeks. Patients with autoimmune blood disorders (mainly immune thrombocytopenic purpura) receive 2 g/kg divided over 2–5 days. Treatment can be repeated every 2 months.

When Lane<sup>5</sup> reported the possibility of NANB hepatitis in hypogammaglobulinaemic patients receiving IVIG manufactured at the Blood Product Laboratory, Elstree,<sup>5</sup> we began a prospective study of clinical and laboratory evidence of viral hepatitis in patients with primary hypogammaglobulinaemia after treatment with Scottish IVIG. Pre-infusion serum samples obtained at each infusion of IVIG were sent for assay of alanine aminotransferase (ALT).

Serial ALT values were available over a period of one year in eleven patients and over 6–10 months in a further five. Nine patients had been established on regular replacement with Scottish IVIG for at least a year before the start of the prospective study. Serum samples taken during that year had been stored at  $-70^{\circ}\text{C}$ , and the pre-infusion samples from these patients were retrieved and examined for ALT activity.

No patient had any clinical evidence of viral hepatitis. Eleven of the sixteen patients had ALT values in the normal range (up to 50

U/l). One patient had chronic liver disease secondary to total parenteral nutrition before the start of IVIG treatment, two patients had two isolated ALT rises (neither above 70 U/l), and ALT values were normal by the next infusion; one patient had a single rise in ALT (60 U/l); and one patient had a transient ALT increase over a 4 week period with a peak value of 91 U/l after 14 months of treatment with Scottish IVIG. All patients were clinically well and other patients who had received the same batches of IVIG did not show any change in serum ALT.

Neither the clinical picture nor the level of serum ALT increase in the above five patients resembles NANB hepatitis or the illness described by Lever, Ochs, and their colleagues.<sup>1,2</sup> Our patients have remained well. Most clinicians will diagnose hepatitis if the ALT level is above the normal range in two or more sequential blood specimens taken 3–17 days apart and if one value is at least twice the upper limit of normal.<sup>6</sup> None of our patients meet these criteria, except for one with chronic liver disease.

Thirty-eight additional patients with various immunohaematological disorders were enrolled in a prospective study in 1984 to assess the clinical efficacy and safety of high doses of the Scottish IVIG. Serial blood samples were taken and tested for ALT before treatment, every week for a month, then 2, 3, and 6 months after treatment. Sixteen patients have completed 6 months' follow-up.

Among the thirty-eight patients, two with viral infections had abnormal ALT levels before treatment. ALT values in samples taken 3 weeks after therapy were in the normal range. Another patient was being treated with androgenic steroids. His ALT was 68 U/l immediately before the first infusion and a peak level of 102 U/l was observed 2 days later. So far he has been followed up for 4 weeks and his ALT remains about 60 U/l. This pattern of ALT elevation is not in keeping with transmission of infection by the immunoglobulin preparation because the peak enzyme activity was within 2 days of the infusion. A fourth patient had a raised lactate dehydrogenase level before IVIG was given and she had an ALT of 114 U/l in a single sample taken 4 weeks after treatment. The ALT level was normal 1 week later. One patient had normal liver-function tests and showed a transient rise in ALT (peak 60 U/l) 4 weeks after therapy. This rise coincided with a course of cyclophosphamide. As in the study of the hypogammaglobulinaemic patients none of these cases showed any clinical evidence to suggest viral hepatitis.

We believe that Scottish National Blood Transfusion Service IVIG does not cause hepatitis. While we have observed some minor increases in serum ALT values of some of our patients, we do not think that these were due to non-A, non-B hepatitis.

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#### NON-A, NON-B HEPATITIS AFTER INTRAVENOUS GAMMAGLOBULIN

SIR,—We note the comments of Dr Brettell (Jan 5, p 51) regarding our report of non-A, non-B (NANB) hepatitis in patients with agammaglobulinaemia after intravenous gammaglobulin.

The first case of hepatitis occurred two weeks after the first

## Sarcoidosis and Primary Hypogammaglobulinaemia: a Report of two Cases and a Review of the Literature

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**Abstract.** We are aware of only four previous cases of Kveim positive sarcoidosis reported in hypogammaglobulinaemic patients and we describe two further patients. Patients with hypogammaglobulinaemia and a sarcoid-like syndrome or sarcoidosis have been reviewed. Similarities between sarcoidosis and a subgroup of late onset primary hypogammaglobulinaemia have been highlighted. It is suggested that sarcoidosis in this patient population is probably commoner than was previously thought, but the association between the two conditions remains unclear.

**Key words.** Sarcoidosis. Hypogammaglobulinaemia. Cellular immunity.

### Introduction

Sarcoidosis is usually associated with normal or elevated serum immunoglobulin levels [1]. The observation of sarcoid-like granulomas in patients with primary hypogammaglobulinaemia has been reported previously [2-12]: but we are aware of only four reported cases where a definite diagnosis of sarcoidosis was confirmed by a positive Kveim test [2, 5, 10, 12]. We would like to report two further cases where sarcoidosis and late onset primary hypogammaglobulinaemia coexisted in the same patient.

### Case reports

Case 1. G.McL. was relatively well apart from recurrent otitis media and sinusitis until 1970 when, at the age of 23, he presented with a febrile illness accompanied with vomiting. A chest x-ray showed bilateral hilar lymphadenopathy. The Mantoux test was negative to 1 unit of tuberculin PPD and a Kveim test was positive. He did not receive any corticosteroid. One year later he was found to have hypogammaglobulinaemia on protein electrophoresis. His sarcoidosis remained active and he continued to have respiratory infections with radiological pulmonary changes. His serum immunoglobulin levels were: IgG 2.0 g/l, IgM 0.12 g/l, and IgA could not be detected. In 1975 he had an acute febrile illness with pleural effusions and E.C.G. abnormalities in keeping with pericarditis. He was treated then with oral corticosteroids and their withdrawal was delayed because respiratory symptoms increased when the dose was reduced.

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In 1976 intramuscular immunoglobulin 750 mg monthly was started with improvement in respiratory symptoms and withdrawal of steroids. In June 1980 following a further attack of pneumonia he developed severe herpes zoster ophthalmicus resulting in temporary paralysis of accommodation and of the fourth cranial nerve. Iridocyclitis and punctate keratitis followed the zoster infection but both resolved eventually with topical therapy. At review in July 1984 he complained of tiredness, weight loss, constant productive cough with mucopurulent sputum and recurrent flu-like episodes. In August 1984, intravenous infusions of immunoglobulin (15 gm) were started and continued at three weekly intervals. By January 1985 he had gained 12 pounds in weight, his cough and sputum were much decreased and he felt fitter. Chest x-ray appearances were unchanged since July 1984 with considerable generalised mottling and coarse reticulation present in the lung fields.

Case 2. R.B. This patient was well until 1978 when he presented with chronic intractable diarrhoea and weight loss. A jejunal biopsy showed partial villous atrophy, *Giardia lamblia* in the lumen and increased lymphocytes with reduced plasma cells in the lamina propria. Serum immunoglobulin concentrations were low (IgG 2.9 g/l, IgA and IgM undetectable). There was slight lymphopenia of  $0.9 \times 10^9/l$  with 91% T cells and 9% B cells [13]; isohaemagglutinins and antibodies to *E. coli* were undetectable; but lymphocyte transformation to phytohaemagglutinin and concanavalin A and tests of neutrophil function were normal [13]. A diagnosis of late onset hypogammaglobulinaemia with complicating chronic giardiasis was made. Despite long courses of metronidazole and mepacrine, weight loss and diarrhoea continued, and therapy was supplemented with immunoglobulin replacement in the form of plasma 15 mg/kg every three weeks. This resulted in symptomatic improvement, increased serum IgG of 6.2 g/l and weight gain of 17 kg.

Four months after the start of his immunoglobulin replacement therapy he was admitted to hospital with diabetic keto-acidosis

(2% glycosuria, ketonuria (+++), blood glucose 31.2 mmol/l; bicarbonate 12 mmol/l). He remained relatively well on twice daily insulin injections until September 1980 when he complained of one month history of drenching night sweats, general malaise, headaches and vague epigastric discomfort. Positive findings on examination were a temperature of 37.5 C and moderate hepatosplenomegaly. Haemoglobin, white cell count and ESR were normal. Liver function tests were slightly abnormal with serum ALT levels ranging between 41 and 67 U/L and Alkaline Phosphatase between 103 and 115 U/L. Chest x-ray and an abdominal lymphangiogram were likewise normal. Stool examination revealed many *Giardia intestinalis* cysts. Mantoux test to 1 unit and 10 units of tuberculin PPD was consistently negative. Throat swabs, early morning urine specimens, mid-stream specimens of urine, blood cultures, skin swabs and marrow cultures were also negative (including alcohol and acid fast bacilli studies). Hepatitis B surface antigen was negative. Liver biopsy and iliac trephine biopsy were normal with no evidence of lymphoma or granuloma formation. No firm diagnosis was reached, but his symptoms settled spontaneously after he received further treatment with metronidazole for giardiasis.

He was admitted again to hospital in 1982 for treatment of a right basal pneumonia. A prominent shadow below the aortic knuckle was noted in his chest x-ray. Repeat chest x-ray a few months later revealed enlargement of hilar lymph glands bilaterally and of the left paratracheal group of nodes without pulmonary infiltration. Hepatosplenomegaly and enlargement of the right scalene node were found on physical examination. Histological examination of the right scalene node biopsy showed numerous non-caseating granulomas with giant cells. The appearances were consistent with sarcoidosis. A Kveim test was performed 18 months later to seek further evidence for sarcoidosis and was positive. T and B lymphocyte numbers were normal but lymphocyte transformation with phytohaemagglutinin, concanavalin A and pokeweed mitogen were severely depressed whereas they were completely

normal previously. There is no family history of sarcoidosis or of insulin dependent diabetes, or of increased susceptibility to infection.

## Comments

It is quite possible that sarcoidosis and hypogammaglobulinaemia developed simultaneously in the first patient although serum immunoglobulin levels were not measured until one year after sarcoidosis was diagnosed. The earlier history of recurrent otitis media and sinusitis would suggest that the primary hypogammaglobulinaemia may have preceded sarcoidosis. The second patient is very unusual in that he developed insulin dependent diabetes mellitus in the total absence of serum autoantibodies and subsequently suffered from sarcoidosis. The clinical features that are consistent with the diagnosis include hilar lymphadenopathy, splenomegaly, non caseating granulomas in the scalene node, non reactive tuberculin test, depressed lymphocyte proliferation and a positive Kveim test. The only unusual finding is the very low level of serum immunoglobulins consistent with primary hypogammaglobulinaemia. The defect in his lymphocyte functions in vitro is consistent with both common variable hypogammaglobulinaemia [14], and sarcoidosis [15].

## Review of literature

Although 85% of patients with sarcoidosis have increased levels of serum IgG, a syndrome resembling sarcoidosis with hypogammaglobulinaemia has been described previously. Prasad first reported in 1954 the case of a 30 year black woman with hypogammaglobulinaemia, hypersplenism, and numerous non caseating granulomas involving lymph nodes, liver and spleen. Although no Kveim biopsy was performed, Zinneman, when reviewing the same patient in his series of 3 patients with hypogammaglobulinaemia thought that she had sarcoidosis. The first well documented hypogammaglobulinaemia patient with a positive Kveim test confirming sar-

coidosis was by Bronsky, although of the three patients in the MRC series published in 1971, there was one who was reported to have sarcoidosis following a positive Kveim test in 1960. In 1970, Sharma and James found widespread granulomas in the liver and scalene nodes of a patient with hypogammaglobulinaemia. Other features consisted of skin anergy to tuberculin, candida albicans, and dinitrochlorobenzene, and depressed lymphocyte transformation to phytohaemagglutinin. Because of the paucity of clinical features, and the negative Kveim test, Sharma and James concluded that the patient did not have sarcoidosis, and that the associated granulomas might have been due to an unknown immunologic mechanism. Since then there have been numerous reports of sarcoidosis or of a sarcoid-like syndrome in patients with hypogammaglobulinaemia, Table I.

Crofts's patient is particularly interesting in that she had selective IgA deficiency associated with raised serum IgG at the time the diagnosis of sarcoidosis was made, and it was only after splenectomy that she developed severe panhypogammaglobulinaemia and cellular immunodeficiency. Crofts thought that the post operative development of IgG and IgM deficiencies could have been related to splenectomy in an already compromised immune system, although selective IgA deficiency can develop into panhypogammaglobulinaemia [9]. The abnormal mononuclear cellular response would be consistent with sarcoidosis. Interestingly, Seigler diagnosed sarcoidosis in two patients with selective IgA deficiency [16].

## Discussion

Reports in the literature seem to indicate that there may be two different clinical entities: sarcoidosis and a sarcoidlike illness in the hypogammaglobulinaemic population. This is not surprising as many features that are common to sarcoidosis e.g. hypersplenism, granulomas and depressed cellular immunity may be encountered in the patient with hypogammaglobulinaemia [9, 18, 14]. Asherson

**Table I**  
**Clinical features of sarcoid-like syndrome in primary hypogammaglobulinaemia**

AGE (Yrs.)	SEX	AUTHOR	KVEIM	SPLENO-MEGALY	Ig * DEFICIENCY	DEPRESSED CMI **	TUBERCULIN TEST	GRANULOMAS	OTHER FEATURES
30	F	Zinneman et al 1954 <sup>[2]</sup>	—	Yes	Yes	—	—	Spleen, liver nodes	
25	M	Hill 1960 <sup>[5]</sup>	+ve	—	Yes	—	—	Nodes	
39	M	Hill 1963 <sup>[5]</sup>	—	—	Yes	—	—	Nodes	
37	F	Bronsky et al 1965 <sup>[3]</sup>	+ve	Yes	Yes	—	—ve	Skin & nodes	Bilateral hilar nodes
35	F	Hill 1965 <sup>[5]</sup>	—ve	Yes	Yes	—	—	Nodes and liver	
36	M	Davis et al 1970 <sup>[4]</sup>	—	No	Yes	—	—ve	Scalene nodes	Anterior mediastinal mass, hilar and paratracheal nodes, pulmonary nodule. Nodular lymphoid hyperplasia. Skin test positive to Mumps and Streptokinase-Streptodornase.
35	F	Sharma & James 1970 <sup>[6]</sup>	—ve	Yes	Yes	Yes	—ve	Liver	Abnormal skin test to candida albicans dinitrochlorobenzene.
47	M	Seigler 1978 <sup>[16]</sup>	—	—	Selective IgA initially	—	—	—	Hilar nodes, pulmonary mottling, arthritis, diabetes mellitus.
21	F	Seigler 1978 <sup>[16]</sup>	—ve	—	Selective IgA	—	—ve	Liver skin	Renal tubular acidosis, hypercalcaemia, nephrocalcinosis, nephrolithiasis.
30	F	Asherson & Webster 1980 <sup>[9]</sup>	—ve	Yes	Yes	—	—ve	Liver	Hilar nodes, positive tests to DNB.
48	F	Asherson & Webster 1980 <sup>[9]</sup>	—ve	—	Yes	—	—ve	Transbronchial biopsy	Reticular pattern in lung fields. Positive skin test to DNB.
?	M	Asherson & Webster 1980 <sup>[9]</sup>	—ve	—	Yes	—	—ve	Transbronchial biopsy	Asthma, positive skin test to DNB.
16	M	Perks & Petheram 1978 <sup>[7]</sup>	—ve	Yes	Yes	Possible clinically	—ve	Skin, lung, pleura & liver	Eyes involvement.
25	F	Perks & Petheram 1978 <sup>[7]</sup>	—ve	Yes	Yes	Yes	—ve	Scalene nodes	Raised serum angiotensin converting enzyme.
43	M	Edelstein et al 1978 <sup>[8]</sup>	—	Yes	Yes	Probably	—ve	Nodes, spleen	
23	F	Crofts et al 1983 <sup>[10]</sup>	+ve	Yes	Selective IgA initially	Yes	—ve	Spleen, liver nodes	IgA deficiency preceding pan-hypogammaglobulinaemia. Repeat Kveim normal later.
12	F	Friedman et al 1983 <sup>[11]</sup>	—	Yes	Yes	Yes	—ve	Liver, spleen	Negative skin test to candida streptodornase, honeycomb pattern in lung fields, decreased serum C4; hypersplenism.
72	F	Lee 1984 <sup>[12]</sup>	+ve	Yes	Yes	—	—	Skin	Leucopenia, haemolytic anaemia, uveitis, raised serum angiotensin converting enzyme.
33	M	Present Case	+ve	Yes	Yes	Yes	—ve	Scalene node	Diabetes Mellitus.
23	M	Present Case	+ve	No	Yes	—	—ve	—	Severe Herpes Zoster with iridocyclitis.

\* IgG, IgA and IgM except where otherwise specified.  
 Figures in brackets represent reference number.

\*\* CMI = cell mediated immunity.



noted splenomegaly in 55% in his series of 40 patients with common variable hypogammaglobulinaemia and 3 patients in the same series had sarcoid granulomas. Sarcoid like granulomas in patients with hypogammaglobulinaemia may represent an abnormal interaction between macrophage and indigestible antigen possibly because of the underlying immune defect [17].

Cunningham-Rundles [14] demonstrated that some patients with late onset primary hypogammaglobulinaemia have a profound loss of both B and T lymphocyte function as studied in in vitro lymphoproliferative assays. Indeed, most of the patients in this review showed evidence of negative tuberculin reaction and 5 patients at least [6, 7, 11, and the two present cases] had documented evidence of abnormal in vitro T cell transformation. Whether this is also needed for granuloma formation in a positive Kveim test in addition to the underlying defect in the humoral system is unclear.

The Kveim test is widely accepted as a reliable and clinically useful aid to the diagnosis of sarcoidosis [19]. False positive reactions are rare, and as low as 1-2% [20, 21] and a positive test can be regarded as virtual proof of active sarcoidosis. However, a positive test can only be obtained in 75% of cases with clinical evidence of sarcoidosis [22]. A negative Kveim does not exclude a diagnosis of sarcoidosis, if the clinical features are typical of the syndrome. It can therefore be argued that sarcoidosis in patients with hypogammaglobulinaemia is commoner than is currently believed, and that nearly all the patients in this review with non reactive Kveim had either a clinical syndrome similar to sarcoidosis or had an atypical

form of sarcoidosis. Nonetheless, until these two conditions are better understood, the association between sarcoidosis and hypogammaglobulinaemia must remain obscure.

### Acknowledgments

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## Immune Hepatitis B Plasma Procurement

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**Abstract.** The requirement for hepatitis B immunoglobulin for passive immunisation persists despite the availability of a safe hepatitis B vaccine, and there is therefore a need for immune plasma for immunoglobulin production. We have screened a large number of blood donors to identify potentially suitable hepatitis B immune plasma donors. The logistics of this have been examined, and the programme was found to be inefficient and labour-consuming. A suitably designed programme of active immunisation with hepatitis B vaccine should provide a more effective means of obtaining plasma.

### Introduction

Plasma with high titres of antibody against hepatitis B (HBsAb) for the manufacture of hepatitis B immunoglobulin (HBIG) is normally collected from suitable donors who have been previously exposed to hepatitis B infection. Our present plasma procurement system relies on large-scale screening of the blood donor population to find suitable donors to be recruited for plasmapheresis. In a previous communication [1], we have found that the prevalence of hepatitis B antibody in the Scottish population is quite low and that screening of the general blood donor population is a large exercise which is time- and labour-consuming. Furthermore, the serum level of antibody to hepatitis B surface antigen is not always maintained, leading to a loss of hepatitis B immune plasma donors,

and as a consequence such screening exercises may have to be repeated at regular intervals. We report the results of such a screening programme in South East Scotland and examine its effectiveness as a means of identifying high-titre donors suitable for plasmapheresis.

### Materials and Methods

#### *Study Design*

Between June and October 1983 all blood donations collected from the normal volunteer blood donor population by the Edinburgh and South East Scotland Regional Blood Transfusion Centre were screened for the presence of hepatitis B surface antibody (HBsAb). HBsAb was subsequently quantified in the positive samples.

Donors with HBsAb levels of 10 IU/ml or greater were identified and approached with a view to recruiting them for plasmapheresis.

The criteria for suitability for plasmapheresis were: (1) serum HBsAb level of 10 IU/ml or greater; (2) age less than 50 years; (3) minimum body weight of 50 kg; (4) good venous access; (5) no major illnesses; (6) absence of HBsAg; (7) availability.

The recruited donors were plasmapheresed at intervals varying between once every 4 and once every 12 weeks, and the volume of each plasma donation was recorded by weighing. Before each plasmapheresis, quantitation of HBsAb level was carried out and liver function tests and absence of hepatitis B surface antigen were monitored.

#### Biochemical Assessment

Venous blood samples were collected at each plasmapheresis for liver function tests by Sequential Multiple Analysis with computer system SMAC 2.

#### Screening and Quantitation of HBsAb Level

Screening of the blood donations was carried out using a solid-phase sandwich immunoassay adjusted to detect samples with hepatitis B antibody level above 1 IU/ml [1]. The antibody level in positive sera was measured using AUSAB (Abbott Laboratories, Chicago, Ill.) radioimmunoassay. A laboratory standard, calibrated against a WHO standard obtained from the Amsterdam Red Cross Central Laboratory, was diluted in 0.1 M Tris-HCl, pH 7.5, containing 50% normal human serum (HBsAg/anti-HBs-negative) to values of 0.32, 0.16, 0.08, 0.04, 0.02, and 0.01 IU/ml. Anti-HBs-containing sera were diluted 1:10, 1:100, 1:1,000, and 1:10,000. Count rates were automatically processed by an NE 1612 gamma counter (Nuclear Enterprises, Edinburgh, Scotland) programmed to construct a standard curve against which to compare appropriate test serum dilutions.

#### Assessment of Hepatitis B Status

Venous blood samples were assayed for hepatitis B surface antigen by radioimmunoassay (Blood Products Laboratory, Elstree, UK).

### Results

Of the 29,265 serum samples that were screened between June and October 1983, 129 samples contained more than 1 IU/ml of HBsAb. Subsequent quantitation of these

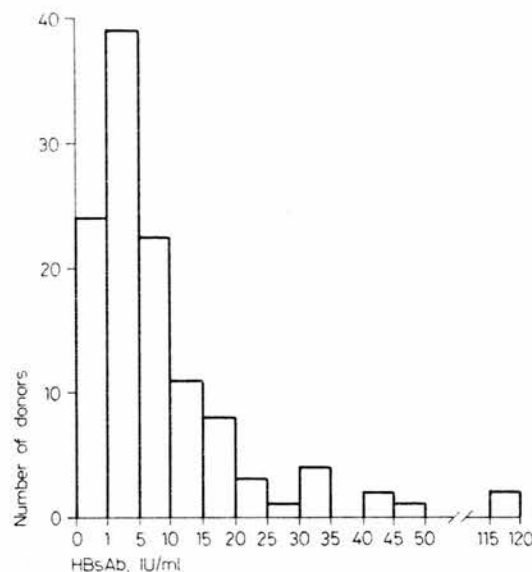


Fig. 1. HBsAb level distribution.

129 positive serum samples showed the distribution according to figure 1.

A total of 19 donors had HBsAb levels of 10 IU/ml or greater with a range of 10.0–116.0 (mean 15.2) IU/ml. Of these 19 newly identified donors that were approached, 10 agreed to be recruited for regular plasmapheresis. One donor had to be withdrawn because routine liver function tests showed evidence of chronic liver disease. Twelve months after the screening programme only 2 out of the newly recruited 9 were still donating hyper-immune hepatitis B plasma. Of those lost, 5 moved elsewhere, and in 2 cases the HBsAb level had fallen and remained consistently below 10 IU/ml.

No donor developed abnormal alanine aminotransferase (ALT) values during the study.

Table I. Screening campaigns carried out in Edinburgh

Date of screening	Number of donations screened	Number (%) of positive donations		Number (%) suitable for plasmapheresis		Number already plasmapheresed	Number recruited for plasmapheresis
		n	%	N	%		
May 1980 to August 1980	10,095	29	0.28	0	0	0	0
October 1980 to December 1980	11,849	47	0.39	3	0.025	0	1
February 1981 to May 1981	16,231	58	0.35	4	0.025	0	0
May 1981 to September 1981	3,210	10	0.31	1	0.031	0	1
September 1981 to August 1982	28,384	46	0.16	8	0.028	1	5
June 1983 to October 1983	29,265	129	0.67	26	0.088	7	9

#### Quantity of Plasma Collected

A total of 30.5 litres of hyperimmune hepatitis B plasma was collected from the 9 newly recruited donors over the 12 months following recruitment (0.8–6.5 litres per donor). The mean volume of plasma per donor collected was 3.4 litres. A total of 424,380 IU of HBsAb was collected with a mean plasma concentration of 13.9 IU/ml of HBsAb.

#### Discussion

Hepatitis B immunoglobulin is used in the prevention of hepatitis B infection following

accidental exposure to the virus and in infants born to mothers who are chronic carriers of the virus. The requirement for this immunoglobulin is likely to continue or increase in view of the increasing incidence of hepatitis B infection [2] despite the availability of a safe hepatitis B vaccine. As a result, we developed a low-cost radioimmunoassay for the screening of donors [1] and we have analyzed the long-term value of random screening of donors for high-titre HBsAb plasma. Of all the donors identified following the screening of 29,265 blood donations, only 2 were still donating regularly 12 months after they had been recruited.

Our experience in recent years is that every year on average a third of donors from the HBsAb plasmapheresis panel become unsuitable because of falling antibody level, or because they have moved elsewhere. Therefore, to maintaining a constant panel of say 15 donors it would be necessary to repeat the screening programme at less than 3-yearly intervals. This present system therefore appears to be a very inefficient way of identifying and recruiting hepatitis B plasma donors.

In previous years, five other screening campaigns had been carried out in Edinburgh: the very poor recruitment confirms our past experience (table I).

We believe that it is of great importance that other methods for procuring HBsAb plasma for the manufacture of HBIg be examined. In particular, now that a safe hepatitis B vaccine is available, the immunisation of suitable donors should be considered. However, while it is generally very difficult for individuals to achieve high enough antibody levels suitable for plasmapheresis (greater than 10 IU/ml) following a primary course of immunisation with hepatitis B vaccine [3, 4], preliminary studies in the North London Blood Transfusion Service have demonstrated that high enough antibody levels can be reached in individuals who have pre-existing naturally acquired hepatitis B antibody by boosting. A consistent 10-fold rise and in certain cases a 60-fold rise in antibody levels following boosting with the Merck, Sharp & Dohme hepatitis B Vaccine has been demonstrated [5]. Therefore, we suggest that

boosting individuals with pre-existing naturally acquired hepatitis B antibody be examined as a means of recruiting suitable hepatitis B immune plasma donors.

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# Serum ALT Levels in Patients with Primary Hypogammaglobulinaemia Receiving Replacement Therapy with Intravenous Immunoglobulin or Fresh Frozen Plasma

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**Abstract.** The intravenous immunoglobulin (IV. IgG) preparation used in this study is manufactured by the Scottish National Blood Transfusion Service (SNBTS) by the pH 4/mild pepsin method. Recent reports suggest that non-A, non-B hepatitis may be transmitted by certain intravenous immunoglobulin preparations. Serum ALT levels were therefore measured prospectively in 16 patients with primary hypogammaglobulinaemia who received an intravenous immunoglobulin replacement therapy (SNBTS IV IgG) over a period ranging from 6 to 25 months. Retrospective analysis of serum ALT levels was also carried out in 8 patients with primary hypogammaglobulinaemia who received fresh frozen plasma (FFP) for periods ranging from 8 months to 13 years. There was no evidence of non-A, non-B hepatitis transmission by either SNBTS IV IgG or by FFP in all the patients studied.

## Introduction

Immunoglobulin preparations generally have a good record for safety with respect to the transmission of viral hepatitis [1]. However, there have been reports of non-A, non-B (NANB) hepatitis in patients with primary hypogammaglobulinaemia receiving intravenous immunoglobulin (IV IgG) preparations that have been treated so as to make them safe for intravenous use [2–4], raising the possibility that NANB hepatitis viruses may not always be inactivated or removed during the manufacture of these immunoglobulin preparations. We report the results of a retrospective evaluation of serum ALT levels in patients with primary hypogammaglobulinaemia receiving an immunoglobulin replacement in the form of fresh frozen plasma (FFP), and a prospective evaluation of serum ALT levels in such patients receiving intravenous immunoglobulin treated by mild pepsin proteolysis at pH 4. Part of this data has been presented in a preliminary communication [5].

## Patients, Materials and Methods

### Patients

All 17 patients had proven primary hypogammaglobulinaemia. Four (cases 8, 9, 10 and 15) were X-linked and the remainder had late onset primary hypogammaglobulinaemia. Sixteen of the preceding patients who were receiving IV IgG were enrolled in the prospective trial. The patients' ages ranged from 11 to 49 years.

### Fresh Frozen Plasma

Plasma was collected for each patient by plasmapheresis of 2 or 4 dedicated donors to minimise the risks of transmitting infection including NANB hepatitis. A dose of 15 ml/kg body weight at 2–4 weekly intervals was used. Plasma donors met the standard medical and serological requirements for blood donors including normal serum ALT levels. The duration of FFP replacement therapy in the 8 patients in the retrospective study ranged from 8 months (case 8) to 13 years (case 14).

### IV IgG Preparation

The IV IgG preparation used in this study is prepared at the Protein Fractionation Centre of the Scottish National Blood Transfusion Service (SNBTS) from a cold ethanol (Cohn) fraction II of plasma collected from volunteer Scottish blood donors. The cold ethanol fraction II is subsequently ultrafiltrated, treated by mild pepsin proteolysis at pH 4 and freeze-dried [6, 7]. The product after reconstitution with water is a solution of 5% (5g/100 ml) human normal immunoglobulin. Patients received 200–400 mg/kg of SNBTS IV IgG every 2–5 weeks. The duration of SNBTS IV IgG treatment ranged from 6 to 25 months.

### Other Immunoglobulin Preparations

Seven of the patients in the study received other intravenous immunoglobulin preparations (Gamimune, Cutter Laboratories; Sandoglobulin, Sandoz) in an earlier evaluation of immunoglobulin therapy [8], and 13 of the patients received standard intramuscular

immunoglobulin manufactured by Cohn fractionation at the SNBTS Protein Fractionation Centre, prior to the study period (fig. 1).

#### ALT Assays

The majority of the ALT levels was measured either by an enzymatic (kinetic) method on a Multistat III centrifugal analyser using a Boehringer ALT (OPT) kit or by sequential multiple analysis with Computer System (SMAC 2), Technicon Instrument Association. In a small number of instances, ALT levels were measured at the patients' local hospital clinical chemistry laboratories using standard automated methods. The normal range of serum ALT levels is 11–50 U/l.

#### Hepatitis B Serology

Venous blood samples were tested for hepatitis B surface antigen by radioimmunoassay prior to the first SNBTS IV IgG infusion. Patients were retested not less than 6 months after the first infusion and in certain cases again at 6–12 monthly intervals. Blood samples were also tested for antibodies to hepatitis B surface, core and 'e' antigens.

#### Study Design

Serum ALT values in the patients who had received FFP therapy were obtained over periods of 6–29 consecutive months in 8 patients. The case records were examined first, and where no ALT results were recorded, pre-infusion serum samples stored at  $-150^{\circ}\text{C}$  in the vapour phase of liquid nitrogen were retrieved for ALT assays.

All 16 patients receiving SNBTS IV IgG had serum samples taken before and after each infusion of immunoglobulin for estimation of immunoglobulin and ALT levels. The following serum samples were available for analysis:

(a) from December 1983, ALT levels in all serum samples were measured prospectively; (b) before December 1983, ALT levels were obtained retrospectively from the patients' case records; (c) where there were no records of the serum ALT having been determined previously, pre-infusion serum samples (stored frozen at  $-150^{\circ}\text{C}$ ) were retrieved and ALT levels measured.

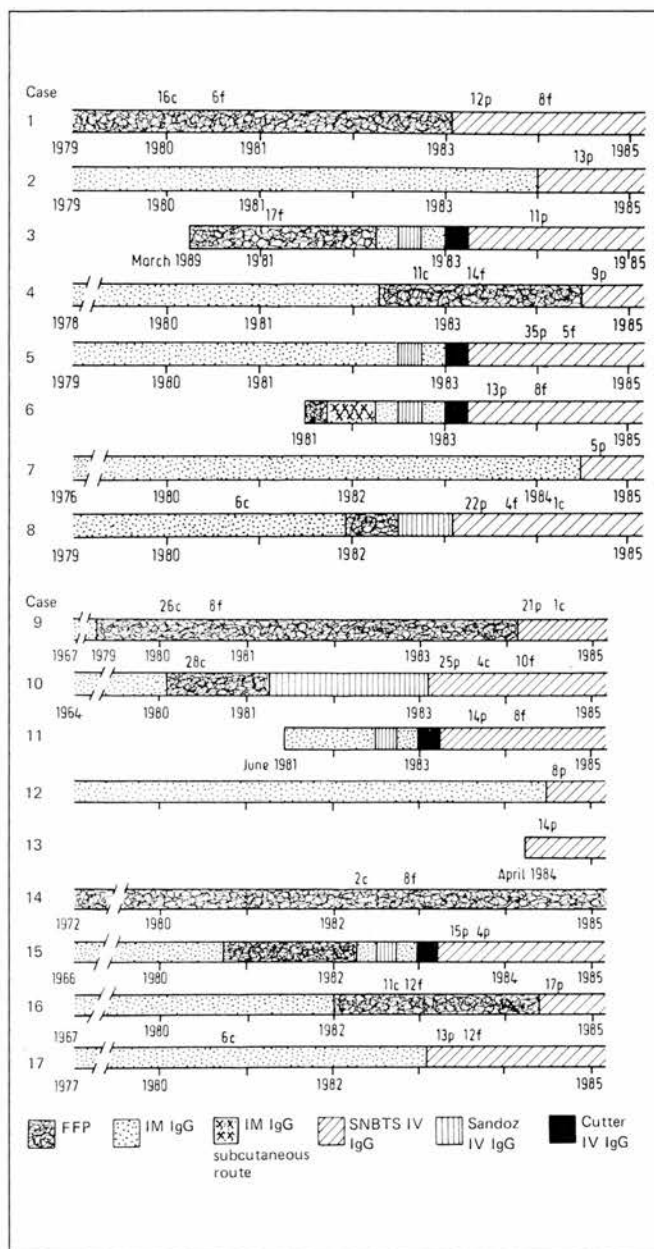
Prospective data on ALT levels were therefore available in 11 patients for 13–15 consecutive months and in a further 5 patients for 5–11 consecutive months.

Blood samples from 7 patients (cases 1, 3, 6, 8, 9, 10, 11 and 14) were collected in Edinburgh, and the sera were separated within 24 h after collection. Blood samples from 9 patients (cases 2, 4, 5, 7, 12, 13, 15, 16 and 17) were collected outside Edinburgh, as these patients received their immunoglobulin infusion at their local hospital and the blood samples were sent by post; the sera were separated immediately on arrival (usually within 24–72 h of collection) and kept at  $4^{\circ}\text{C}$  until assay.

A patient was diagnosed as having hepatitis if the ALT was above the normal range (50 U/l) in two or more sequential blood samples taken within a 3- to 17-day interval, and if one of these levels was at least twice the upper limit of normal (100 U/l). An episode of hepatitis was considered to be probably of viral aetiology if there was no other identifiable cause for the raised ALT [9].

#### Validity of Stored Samples

Serum was collected from patients with liver disease of non-infective aetiologies and sent for ALT assay. Aliquots of the sera were stored at  $-20^{\circ}\text{C}$  and at  $-150^{\circ}\text{C}$  for at least 6 months before they were assayed for ALT activity.

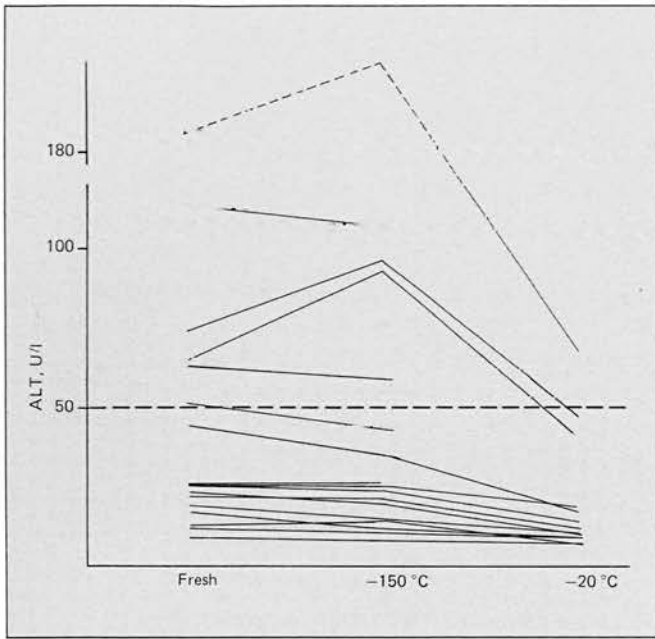


**Fig. 1.** Types of Immunoglobulin in replacement therapy received by cases 1–17. The numbers above the hatched lines indicate the number of samples in which ALT levels were determined. c = ALT values obtained from patients' case notes; f = ALT values obtained on samples stored at  $-150^{\circ}\text{C}$ ; p = ALT values obtained in prospective study. Sandoglobulin and Gamimune are indicated as Sandoz IV IgG and Cutter IV IgG, respectively.

## Results

#### Validity of the Stored Sera

ALT values of samples stored for at least 6 months at  $-20^{\circ}\text{C}$  and at  $-150^{\circ}\text{C}$  were compared with the ALT values



**Fig. 2.** Serum ALT values of samples in which ALT levels were determined immediately after collection or after storage at  $-150^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$  for 6 months. The horizontal broken line indicates the upper limit of the normal range for serum ALT values; the diagonal broken line indicates changes in ALT levels in a sample with an initial ALT value of 187 U/l.

measured at the time of sample collection. It was found that serum ALT activity decreased after storage at  $-150^{\circ}\text{C}$  and at  $-20^{\circ}\text{C}$  for up to 6 months. The decline in ALT activity was minimal for samples stored at  $-150^{\circ}\text{C}$  in contrast to samples stored at  $-20^{\circ}\text{C}$ , when significant decreases in serum ALT activity were noted (fig. 2).

#### *ALT Levels during FFP Therapy*

Among the 8 patients who had received FFP therapy in the study, 100 serum ALT levels were available from the case notes: 25 samples (from 3 patients) had levels above 50 U/l, 2 from case 1 (67 and 55 U/l), 7 from case 9 (range 53–113 U/l) and 16 from case 10 (range 52–203 U/l). From an additional 65 frozen serum samples sent for ALT measurement, only 3 were abnormal, all from case 1 (114, 60 and 69 U/l). Therefore, of 165 serum ALT values available for analysis, 28 from 3 patients) were abnormal (fig. 3a, e, f).

#### *ALT Levels during SNBTS IV IgG Therapy*

A total of 249 serum samples were obtained and assayed for ALT levels immediately prior to each infusion. All but 7 were in the normal range (10–50 U/l). The 7

samples with abnormal values were from 4 patients: case 3 (91 and 53 U/l), case 9 (60 U/l), case 15 (70 U/l) and case 17 (96, 84 and 234 U/l) (fig. 3b, e).

An additional 60 serum samples were retrieved from  $-150^{\circ}\text{C}$  storage and examined for ALT activity. Only 4 were abnormal, 2 from case 1 (54 and 69 U/l) and 2 from case 5 (75 and 58 U/l).

A further 5 normal ALT values were noted on review of the case notes.

Therefore, of a total of 314 serum ALT values, 11 (from 6 patients) were above the upper limit of normal (50 U/l).

Hepatitis B surface antigen was negative in all the specimens tested.

#### *Relationship of raised ALT Values to the Clinical Condition of the Patients*

**FFP Therapy.** Three out of eight patients studied developed raised serum ALT values during FFP therapy that could be consistent with NANB hepatitis. However, in each case there were more probable explanations for the abnormal ALT. One patient (case 1) was diagnosed as suffering from sarcoidosis which was active at the time the ALT was raised [19]. Another patient (case 10) was clinically unwell with recurrent episodes of septicaemia and had a cholestatic pattern of liver function tests (alkaline phosphatase of 3,351 U/l). The last patient (case 9) had multiple problems associated with hypogammaglobulinaemia including severe malabsorption with weight loss and recurrent chest infections.

**SNBTS IV IgG.** Out of a total of 314 blood samples, 11 abnormally raised ALT values were observed occurring in 6 of the 16 patients during SNBTS IV IgG therapy. No markers of hepatitis B infection were detected in the patients at any time.

Figures 3c, d and g illustrate 3 patients (cases 4, 8 and 16) with normal serum ALT levels during therapy with FFP and SNBTS IV IgG.

Further details of the 6 patients (cases 1, 3, 5, 9, 15 and 17) who developed raised ALT levels are shown below.

**Case 1** had raised serum ALT levels during FFP therapy (see above), secondary to active sarcoidosis, and it is likely that the two raised serum ALT values (54 and 69 U/l) were the result of his sarcoidosis.

**Case 3** had received SNBTS IV IgG since April 1983. In April 1984, before her 18th infusion of SNBTS IV IgG, she was found to have a high serum ALT of 91 U/l while remaining clinically very well. Three weeks later her serum ALT was 44 U/l. No cause for the raised ALT was found, and in particular there was no history of drug or



alcohol ingestion; hepatitis B surface antigen as well as other virological markers of hepatitis were absent.

Case 5 received Sandoglobulin and Gamimmune for 3 months each prior to the study period. His hypogammaglobulinaemia is associated with malabsorption secondary to villous atrophy, frequent respiratory and gastrointestinal infections requiring treatment with numerous antibiotics including erythromycin for intractable *Campylobacter enteritis*. Two abnormal ALT values (75 and 58 U/l) were observed when the frozen samples were analysed. Retrospective analysis of the case records suggest that he was not unwell at the time of the abnormal ALT levels and that there was no apparent cause for the abnormal ALT.

Case 9 had documented abnormal serum ALT values during FFP therapy. At the time of the single abnormal ALT level (60 U/l) which followed his 15th SNBTS IV IgG infusion, he was asymptomatic.

Case 15 was diagnosed in 1966. After years of treatment with intramuscular gammaglobulin he received FFP followed by Sandoglobulin and Gamimmune (for 3 months each) before treatment was commenced with SNBTS IV IgG in April 1983. A single raised ALT value (70 U/l) was noted in February 1984. Four days elapsed between the collection of the sample and the separation of the serum, and all subsequent serial ALT values have been normal. An additional factor in this case is that the attending physician reports that this patient has a large regular alcohol consumption.

Case 17 had been on total parenteral nutrition since 1980 because of intractable diarrhoea with severe malabsorption. His medical history included chronic cholecystitis and cholelithiasis treated by cholecystectomy in 1980 and since 1981 had documented abnormal liver function tests including raised ALT ranging from 50 to 234 U/l. A liver biopsy in 1980 showed mild chronic portal triaditis with occasional granulomatous foci. A repeat liver biopsy in 1984 had similar features, and in particular there were no features to suggest NANB hepatitis. His abnormal liver function tests were considered by his gastroenterologist to be a consequence of his underlying disorder and of an excess intralipid content in his total parenteral nutrition regime [10, 11].

## Discussion

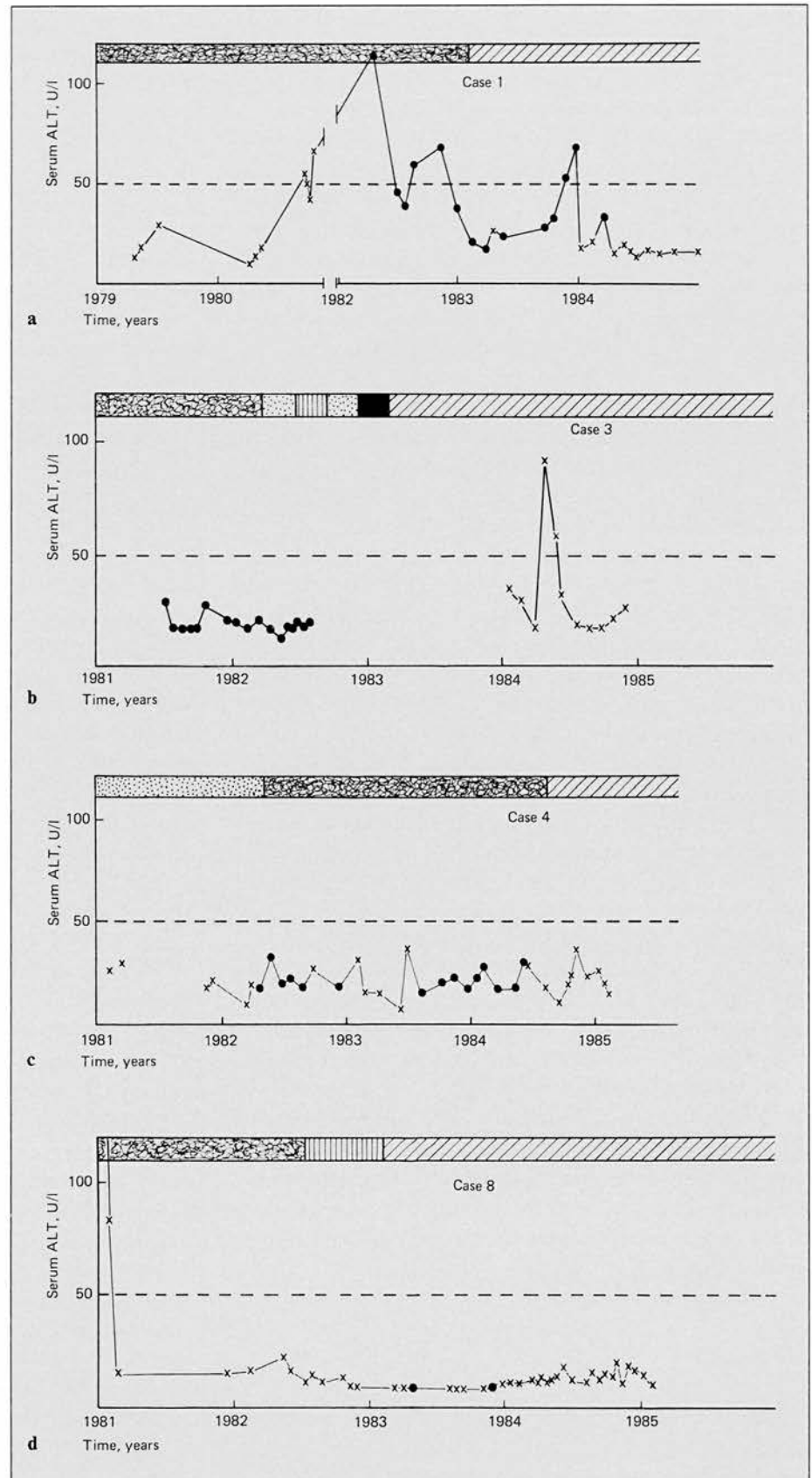
Many serum enzymes may remain stable for some time when stored at  $-20^{\circ}\text{C}$ . Serum ALT has also been shown to be stable for 3–4 days at room temperature and up to 7

days if kept refrigerated at  $4^{\circ}\text{C}$  [12, 13]. Our data suggest that while there seems to be a fall in serum ALT activity after storage at  $-150^{\circ}\text{C}$  for 6 months, the fall is minimal in contrast to a storage at  $-20^{\circ}\text{C}$  (fig. 2). It also seems unlikely that any serum sample with significantly raised ALT values (100 U/l or greater when considering hepatitis) would have been missed because they had been stored at  $-150^{\circ}\text{C}$  for 6 months.

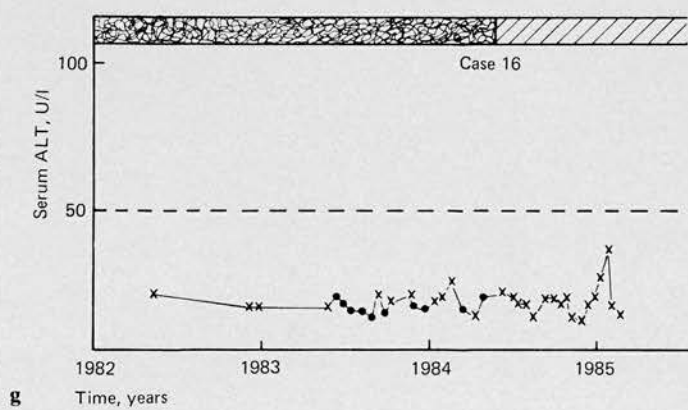
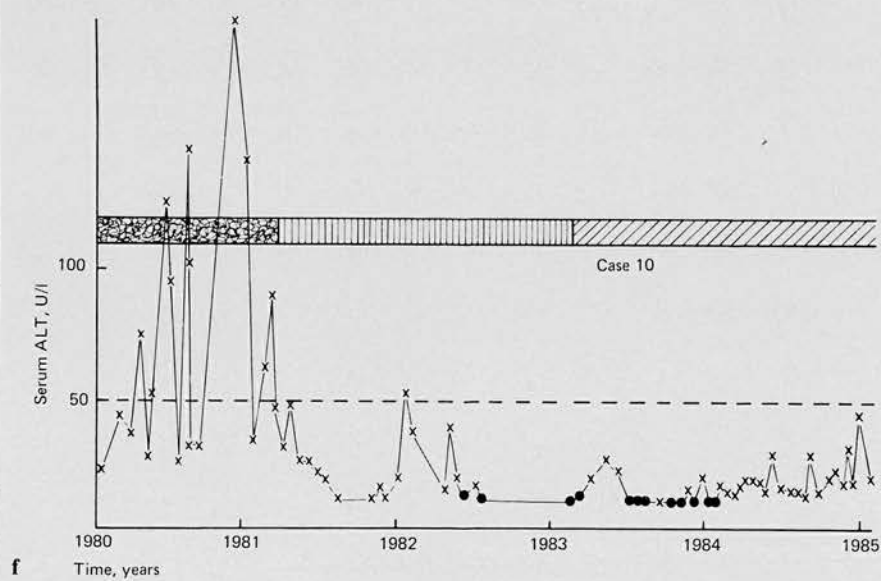
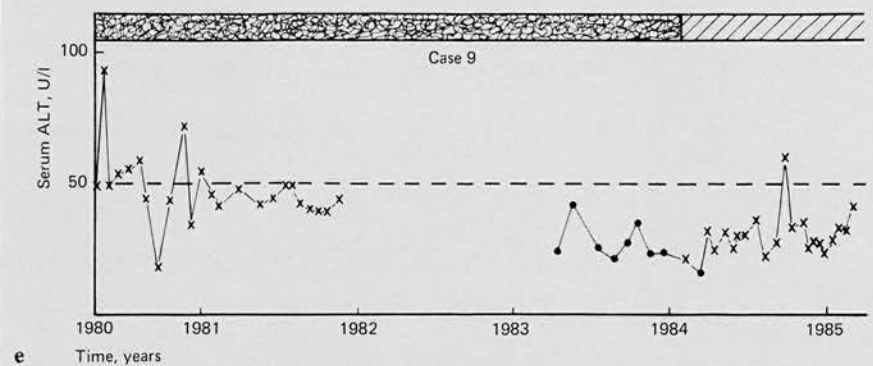
Three of our patients receiving FFP have been noted to have raised serum ALT levels. Although the magnitude of their serum ALT rise is consistent with hepatitis, there has been an obvious cause for their abnormally raised ALT; 1 had active sarcoidosis, and the remaining 2 were very ill with infection including recurrent septicaemia in 1 patient. In addition, there were no features to suggest NANB hepatitis.

None of our patients during SNBTS IV IgG therapy would fulfil the criteria for hepatitis, except for the patient with known liver disease. Only a small proportion of patients receiving regular IV IgG infusions had any ALT abnormalities. The pattern and magnitude of disturbance of liver function tests was not that of NANB hepatitis. In 5 of the 6 patients with raised ALT levels during IV IgG treatment there appears to be an explanation other than NANB hepatitis. Only 1 patient had an unexplained elevated ALT level (peak ALT of 91 U/l) which became normal within 3 weeks, but by definition [9] she did not have hepatitis and was clinically well throughout. Furthermore, neither the clinical picture nor the level of serum ALT rise in these patients resemble that of NANB hepatitis or the illness described by *Lever and Ochs* [2, 3].

Conventional immunoglobulin preparations virtually never transmit viral infections [14–16]. However, several recent reports [2, 3] indicate that some immunoglobulin products designed for intravenous administration may not be as safe as the conventional intramuscular product. It is probable that production methods influence the safety of intravenous preparations in terms of transmission of viral infections. In particular, the presence or absence of further processing to reduce the level of spontaneous anti-complementary activity and of vasoactive enzymes following the removal of ethanol may be important. Not all of these procedures are thought to be virucidal. Neither of the two intravenous preparations known to have transmitted NANB hepatitis [2, 3] were subjected to a recognised virucidal finishing treatment, e. g. acid treatment (pH 4.0) or Beta-propiolactone and ultraviolet irradiation [17]. Our data show that SNBTS IV IgG which is processed for intravenous use by the pH 4/pepsin



**Fig. 3a-g.** ALT values in 7 patients receiving different types of immunoglobulin therapy. ALT values were either determined in samples assayed within 72 h of collection (X) or after storage at  $-150^{\circ}\text{C}$  (●). The horizontal broken line indicates the upper limit of the normal range for ALT levels; the hatched horizontal areas indicate the type of immunoglobulin therapy received by the patient (key identical to fig. 1).



method [18] and FFP obtained from 2–4 properly screened dedicated donors do not transmit NANB hepatitis.

### Acknowledgements

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## CASE REPORTS

### Salmonellosis in patients with primary hypogammaglobulinaemia

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#### Summary

Three of 20 patients with primary hypogammaglobulinaemia developed *Salmonella* gastroenteritis. Two of them who also had low concentrations of serum IgG and pernicious anaemia were clinically septicemic. Patients with primary hypogammaglobulinaemia have previously been thought not to be more susceptible to *Salmonella* infection but a combination of low gastric acidity and impaired humoral immunity may predispose them to such infection.

#### Introduction

Late-onset primary hypogammaglobulinaemia (PH), also known as common variable immunodeficiency, is a rare condition characterised by a deficiency of the three main classes of immunoglobulins and an absence of IgA in intestinal secretions. Affected patients are prone to recurrent bacterial infections. They also have a high incidence of diarrhoeal illness which is not always attributable to an increased susceptibility to infection. More specifically, these patients are not believed to be more susceptible to salmonellosis<sup>1</sup> although they suffer from *Giardia* and other parasitic infestations as well as campylobacter infection.

In 20 patients with late onset PH, we observed an unusually high incidence (three of 20) of salmonella infection.

## CASE REPORTS

### Patient 1

At the age of 28 years, while pregnant in 1975, this patient was found to have hypogammaglobulinaemia because of absent isohaemagglutinins. Her immunological profile is shown in Table I. Treatment began with a small intramuscular dose of immunoglobulin which was increased in 1979 to 4.5 g monthly in divided doses. In 1978, she developed anaemia and malabsorption associated with giardiasis. She remained anaemic despite appropriate treatment and pernicious anaemia was diagnosed by means of the Schilling test. Antibodies

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# Selection of Plasma Donors Suitable for Tetanus Boosting

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**Abstract.** As a result of a comparative trial examining the immunogenicity and clinical reactivity of two tetanus vaccines, 33 donors with high antibody levels were plasmapheresed over 6 months for the collection of tetanus hyperimmune plasma. Serial antibody assay results and the frequency of adverse reactions were analysed to define the donors most suitable for an immune plasma programme. A high proportion of boosted donors were withdrawn from plasmapheresis because of poor persistence of antibody levels. Donors most suitable for boosting appear to be males under 25 years old with a tetanus antibody level of around 8 IU/ml prior to boosting.

## Introduction

Tetanus immune globulin is manufactured from plasma collected from human donors who have high levels of tetanus antibody. These donors are usually selected shortly after receiving a course of tetanus immunization or a tetanus boost following trauma. However, this method of recruiting donors is not entirely satisfactory because not all donors achieve suitably high antibody levels (greater than 10 IU/ml) after immunization. Some plasma-collecting organisations therefore boost selected donors to render them suitable for donating hyperimmune tetanus plasma [1, 2].

Following a study comparing two differently purified tetanus absorbed vaccines in volunteer blood donors [3], 33 donors with high tetanus antibody were plasmapheresed for up to 6 months. The results of the vaccine trial and subsequent plasmapheresis were examined to define the characteristics of donors most suitable for a programme of hyperimmunization with tetanus toxoid. In particular, we examined the factors that influenced the incidence of adverse reactions to tetanus boosting, as well as those that determined the persistence of antibody levels suitable for plasmapheresis.

## Volunteers, Materials and Methods

205 volunteer blood donors (mean age 32.9 years, range 18.7-64.8 years) participated in the trial [3]. The participants were randomised with respect to age, sex and pre-immunization tetanus antibody levels. Following boosting with 0.5 ml of one of the two tetanus vaccines (conventional tetanus vaccine or immune-purified tetanus vaccine), the vaccinees were asked to complete a daily calendar record for 7 days to report the occurrence and severity of local reactions at the site of injection (pain, redness, tenderness and lump), and systemic reactions (headache, feverishness and malaise). Vaccinees also recorded their oral temperature daily for 7 days after immunization.

After the completion of the trial, donors were invited to be plasmapheresed if they fulfilled the following criteria: (1) serum antibody level of 10 IU/ml or greater, (2) age less than 50 years, (3) body weight greater than 50 kg, (4) good venous access, (5) no major illnesses, (6) absence of hepatitis B surface antigen, (7) availability. 36 donors were invited to donate plasma. The frequency of plasmapheresis varied from once every 4 weeks to once every 12 weeks depending on availability. Donors whose tetanus antibody levels dropped below 10 IU/ml on two successive occasions were withdrawn from the plasmapheresis panel.

The serum tetanus antibody levels were measured using an ELISA method shown to correlate well with mouse protection assay [G.R. Barclay, unpublished].

All volunteers gave informed consent to participation in the study, and the protocol was approved by the Ethical Committee of the Scottish National Blood Transfusion Service.

**Table I.** Age and antibody response

Final antibody level, IU/ml	Age, years				
	≤25	25-35	35-50	>50	19-65
0	1 (2)	10 (13)	15 (30)	5 (24)	31
≤10	28 (49)	32 (42)	18 (36)	10 (48)	88
>10	28 (49)	34 (45)	17 (34)	6 (29)	85
Total	57 (100)	76 (100)	50 (100)	21 (100)	204

Figures in parentheses indicate percentage.

**Table II.** Sex and antibody response

Final antibody level, IU/ml	Male	Female	
0	14 (13)	17 (17)	31
≤10	45 (43)	43 (43)	88
>10	46 (44)	39 (39)	85
Total	105 (100)	99 (100)	204

Figures in parentheses indicate percentage.

**Table III.** Antibody increase

	Pre-immunisation antibody level, IU/ml				
	0 (n = 118)	0-2 (n = 33)	2-4 (n = 23)	4-8 (n = 21)	>8 (n = 9)
Number with increase in antibody level after immunisation	89 (75)	31 (94)	22 (96)	20 (95)	7 (78)
Increase in antibody, IU/ml					
Mean	8.76	8.44	9.50	8.08	7.75
± SD	10.60	7.21	6.46	8.87	10.44

Figures in parentheses indicate percentage.

### Statistics

Statistical analyses were carried out using Spearman rank correlation for relationships between two continuous variables and analysis of frequency was carried out by a Yates-corrected chi-square test using a 2x2 table and 1 degree of freedom.

## Results

The two vaccines were found to be similar with respect to the frequency of reactions encountered in the vaccines and to their immunogenicity [3]. For the purposes of data presentation and analysis in this study, we have not made any distinction between the two vaccines.

### *Effect of Age, Sex and Pre-immunization Tetanus Antibody Level on Antibody Level at 2 Weeks after immunisation*

There was a significant correlation between age and antibody level assayed 2 weeks after immunization ( $r = -0.30$ ,  $p < 0.001$ ) and between age and increase in antibody level defined as the difference between post- and pre-immunization antibody levels ( $r = -0.24$ ,  $p < 0.001$ ; table I).

There was no significant sex difference in the magnitude of antibody increase (table II).

The magnitude of antibody increase was independent of the pre-immunization antibody level (table III).

### *Effect of Preimmunization Antibody Level, Sex and Age on Incidence of Reactions to Vaccine*

The sum of the number of days on which the vaccinees experienced any moderate or severe symptoms was used for the analysis of adverse reactions. There was no significant correlation between pre-immunization antibody level and overall reactions; however, the data suggest that there may be an unusually high incidence of reactions amongst vaccinees with antibody levels which were detectable but less than 2.0 IU/ml (table IV). Males had significantly fewer reactions than females ( $p < 0.01$ ; table V), but there was no evidence that age had any effect on the incidence of adverse reactions.

When the individual reactions were analysed, there was a significantly higher incidence of moderate or severe tenderness and redness among females ( $p < 0.01$ ). There was also a correlation between pre-immunization antibody level and pain ( $r = 0.21$ ;  $p < 0.01$ ) and between pre-immunization and frequency of tenderness ( $r = 0.20$ ;  $p < 0.01$ ). The incidence of individual reactions was also analysed in vaccinees grouped according to their pre-immunization tetanus antibody levels. While there was a significantly higher incidence of moderate and severe pain at the injection site in subjects with a pre-immunization antibody level above 8 IU/ml ( $\chi^2 = 4.15$ ,  $p < 0.05$ ), the increased incidence of moderate and severe tenderness in that group was not statistically significant ( $\chi^2 = 3.80$ ,  $p > 0.05$ ) when compared with the group with no detectable pre-immunization antibody level.

**Table IV.** Correlation between pre-immunisation antibody level and reaction to vaccination

Reaction	Pre-immunisation antibody level, IU/ml				
	0 (n = 118)	0-2 (n = 33)	2-4 (n = 23)	4-8 (n = 21)	>8 (n = 9)
<i>Local reactions</i>					
Redness	17 (14)	9 (27)	5 (22)	3 (14)	2 (22)
Pain	3 (2)	1 (3)	2 (9)	3 (14)	2 (22)*
Tenderness	9 (8)	11 (33)**	1 (4)	5 (24)	3 (33)
Lump	55 (47)	17 (52)	12 (52)	13 (62)	6 (66)
<i>Systemic reactions</i>					
Temperature	4 (3)	4 (12)	1 (4)	1 (5)	0
Headache	13 (11)	9 (27)*	3 (13)	2 (10)	2 (22)
Feverishness	2 (2)	4 (12)	1 (4)	2 (10)	1 (11)
No moderate or severe reaction	95 (81)	14 (42)	19 (83)	13 (62)	5 (56)
Some moderate or severe reaction	23 (20)	19 (59)	4 (17)	8 (38)	4 (44)

Figures in parentheses indicate percentage.

\*0.05 > p > 0.025; \*\*p < 0.001.

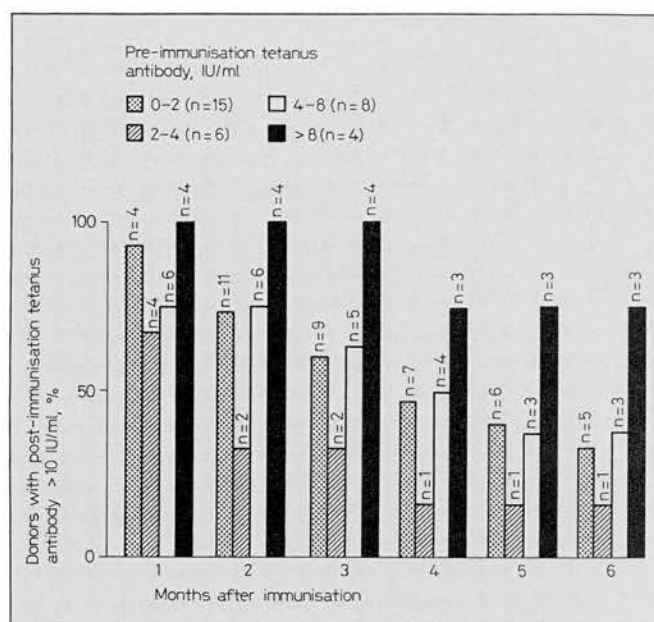
**Table V.** Frequency of reactions among sexes

	Male (n = 105)	Female (n = 99)
<i>Local reactions</i>		
Redness	11 (10)	25 (25)*
Pain	3 (3)	8 (8)
Tenderness	8 (8)	21 (21)*
Lump	46 (44)	57 (58)
<i>Systemic reactions</i>		
Temperature	2 (2)	8 (8)
Headache	15 (14)	14 (14)
Fever	3 (3)	7 (7)
Total reactions (pain, tenderness, fever, headache)	22 (21)	36 (36)*

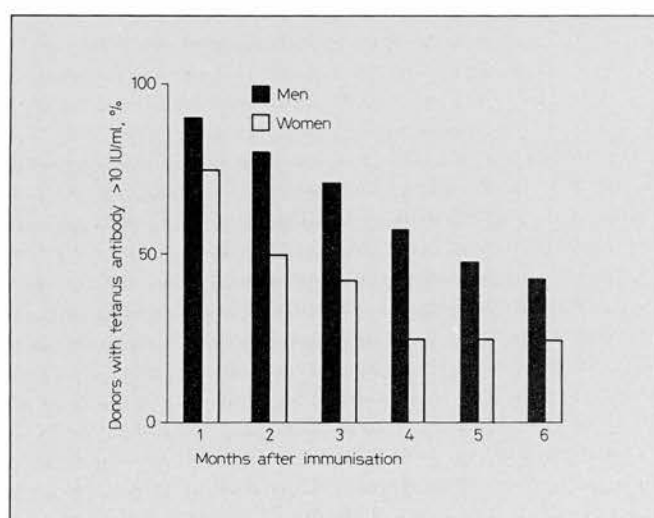
Figures in parentheses indicate percentage.

\*p < 0.01.

Interestingly, the group with detectable pre-immunization antibody levels of less than 2 IU/ml showed the highest frequency of reactions which in many cases were significant as compared to the group without detectable pre-immunization antibody.



**Fig. 1.** Distribution of plasma donors with post-immunization tetanus antibody levels greater than 10 IU/ml who were still donating in the 6 months following immunization. Donors are allocated to groups according to their pre-immunization tetanus antibody levels.



**Fig. 2.** The sex distribution of donors with post-immunization tetanus antibody levels greater than 10 IU/ml during the first 6 months following immunization.

### Volume of Hyperimmune Plasma Collected

36 donors (23 men and 13 women) with high post-immunization antibody levels were recruited for plasma-pheresis. Three donors were withdrawn from plasma-pheresis: 2 men were unavailable to attend regularly



because of business commitments and 1 woman developed a medical condition unrelated to immunization or plasmapheresis. By 6 months after immunization, plasmapheresis had been stopped in 21 of the 33 remaining donors (62%) because their antibody levels had fallen below 10 IU/ml. The volume of plasma collected in the 6 months following immunization was 54.5 litres. This contained a total of 1,201,400 IU of tetanus antibody with a mean antibody concentration 22.0 IU/ml.

#### *Characteristics of Donors with Persistently High Antibody Levels*

There was no clear relationship between the persistence of antibody level above 10 IU/ml and the interval since the last tetanus boost, but 75% of donors with pre-immunization antibody levels above 8 IU/ml appeared to maintain an antibody level above 10 IU/ml after boosting for at least 6 months (fig. 1).

Of the 21 men and 12 women who were regularly plasmapheresed, 9 men (43%), and 3 women (25%) still possessed antibody levels above 10 IU/ml after 6 months of plasmapheresis (fig. 2).

#### **Discussion**

The frequency of unselected blood donors who have a tetanus antibody level above 10 IU/ml is very low, ranging from 1.5% [4] to 2.8% [5] so that random screening is an inefficient method of finding immune plasma donors. A programme of hyperimmunization of plasma donors with tetanus toxoid can overcome the difficulty in recruiting tetanus antibody plasma donors [1, 2]. However, before such a programme is set up, it is important to identify donors who are unlikely to suffer from severe adverse reactions and who can be expected to produce good antibody levels for a long time after boosting.

Our results do not show any statistical difference between the sexes in terms of antibody response to tetanus toxoid. However, the increase in antibody level following boosting with tetanus toxoid was significantly less in those over the age of 40 years. The reduced antibody response with increasing age has previously been observed [6, 7].

It is generally difficult to compare reaction rates between different tetanus vaccine studies because of differences in the vaccinees and in the methods of assessing adverse reactions [8]. Published reports show that women are more susceptible to reactions than men [9, 10], and that there is a higher frequency of reactions in persons

with high levels of pre-immunization tetanus antibody [11, 12]. In this study, we have confirmed that women are more likely to experience adverse reactions than men. We have also found a trend towards a higher frequency of adverse reactions with increasing pre-immunization antibody levels, but this was only significant for moderate and severe pain in the group of vaccinees with a pre-immunization antibody level above 8 IU/ml as compared with the group with undetectable pre-immunization antibody level. Our results also suggest unexpectedly that the highest and most significant incidence of reactions in individuals with pre-immunization anti-tetanus antibodies is in the group with pre-immunization antibody levels between 0 and 2 IU/ml. This finding has not been previously reported. Since this increased frequency was observed equally in recipients of either immunopurified or conventionally purified vaccine [3], it may not be related to vaccine preparation [8]. We favour the interpretation that these reactions may be an Arthus-type phenomenon [9], where it is possible that in this antibody range, the ratio of antibody to antigen in some subjects was such that immune complexes were formed and caused the reactions.

Although among the donors with persistently high tetanus antibody levels (above 10 IU/ml) 6 months after boosting, there was a preponderance of men and of donors with a pre-immunization antibody level above 8 IU/ml, it is difficult to draw any definite conclusion as the numbers are small. Furthermore, it is not surprising that following boosting, individuals with high pre-immunization antibody levels would maintain a high antibody level longer than those with lower pre-immunization antibody levels.

This study confirms that boosting of volunteer donors for the production of plasma containing high levels of tetanus antibody produces only minor reactions and that individuals with high tetanus antibody levels can be hyperimmunized without an excess of adverse reactions. Boosting is therefore appropriate in situations where it is difficult to recruit adequate plasma donors for tetanus antibody. Our data show that the donors most suitable for hyperimmunization with tetanus toxoid are likely to be young males preferably under 25 years of age, with a pre-immunization tetanus antibody level of around 8 IU/ml.

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## Increase of Serum Immunoglobulin Level into the Normal Range in Primary Hypogammaglobulinaemia by Dosage Individualisation of Intravenous Immunoglobulin

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**Abstract.** The effect of varying the dose or the frequency or of both dose and frequency of immunoglobulin administration was evaluated in 17 patients with primary hypogammaglobulinaemia treated with Scottish National Blood Transfusion Service intravenous immunoglobulin (SNBTS IV IgG), an intravenous immunoglobulin preparation manufactured by the pH 4/mild pepsin method. Individualised dosage of SNBTS IV IgG resulted in trough serum IgG levels of greater than 4.0 g/l in 13 of the 17 patients studied. 6 of the 17 patients had trough serum IgG levels in the normal range (5.0 g/l). In 2 patients, more frequent infusions were required to achieve normal trough serum IgG levels because of their clinical conditions. In 1 of these 2 patients, the frequency could be reduced to three weekly when the gastrointestinal loss of IgG was reduced. There was a low incidence of adverse reactions (6.4%) which occurred mainly during the initial infusions. Most patients benefited from an increased serum IgG level, but the frequency of diarrhoea was unaffected. One patient with severe bronchiectasis continued to suffer from respiratory infections despite normal serum IgG levels.

### Introduction

In patients with primary hypogammaglobulinaemia, immunoglobulin replacement therapy has largely been limited until recently to the administration of either intramuscular immunoglobulin or of fresh frozen plasma. The dose of IgG administered to patients with hypogammaglobulinaemia (25–50 mg/kg/week) has been based on the clinical trial held by the MRC [1]. However, the increments in serum IgG level achieved in patients treated with this dose of IgG are highly variable and IgG levels hardly ever rise into the normal range. The intravenous administration of large quantities of immunoglobulin has now been shown to be both safe and well tolerated in the patients with primary hypogammaglobulinaemia [2–4]. As a result, clinicians have attempted to achieve higher serum IgG concentration in their patients with a view to reducing the incidence of infections. Most trials have used larger doses of IgG per infusion but the intervals between infusions have been kept fixed at either 3 or 4 weeks [5–7]. The optimal dose schedule has therefore not been established.

As part of a larger study evaluating the safety and efficacy of a new intravenous immunoglobulin preparation [Leen et al., in preparation], we have tried to individualise the dose of infused IgG in order to increase into the normal range the trough serum IgG concentration of our patients with primary hypogammaglobulinaemia. We report our results based on varying either the dose of IgG infused or the frequency of infusions, or varying both the dose and frequency of IgG infusions.

### Materials and Methods

#### *Patients*

A total of 17 patients (age range 11–50; 11 male, 6 female) (from February 1983 to May 1985) received Scottish National Blood Transfusion Service intravenous immunoglobulin (SNBTS IV IgG) for at least 6 months. The duration of treatment was over 12 months in 13 of the 17 patients, over 18 months in 10 and over 2 years in 8. Twelve suffered from late onset primary hypogammaglobulinaemia (common variable immunodeficiency) and 5 from X-linked primary hypogammaglobulinaemia. All patients had a history of recurrent

Table I. Clinical details of individual patients

Patient	Age at commencement of study years	Diagnosis	Other diagnoses <sup>1</sup>	Clinical outcome <sup>2</sup> (improvement)		IgG at diagnosis g/l	IgG before study g/l	IgG after study g/l	Treatment during 3 months before study
				chest	GI				
1	29	CVI	IDDM	yes	n.a.	2.9	5.47	5.28	FFP 1 litre/3 weeks
2	21	CVI	sarcoidosis	yes	no	1.49	2.5	5.28	IM IgG 1.5g/week
3	25	CVI	NLH	yes	n.a.	1.0	4.34	8.4	IV IgG Gamimune
4	22	CVI	giardiasis						0.2g/kg/3 weeks
5	29	CVI	meningitis						nil
6	41	CVI	–	no	n.a.	2.3	1.53	4.7	FFP 800–1,500 ml/3 weeks
7	29	CVI	bronchiectasis	yes	no	3.0	8.08	4.40	IV Gamimune
8	41	CVI	villous atrophy	yes	no	1.1	2.73	7.2	0.2g/kg/3 weeks
9	19	CVI	ileal strictures	yes	n.a.	0.7	6.24	3.8	IV Gamimune
10	16	CVI	–	yes	n.a.	1.7	0.97	4.05	0.2g/kg/3 weeks
11	19	X-linked	sarcoidosis	yes	n.a.	undetectable	1.46	3.05	IM IgG 750mg/month
12	21	X-linked	NLH	yes	no	undetectable	2.24	4.7	IV Sandoglobulin
13	37	X-linked	bronchiectasis	yes	no	1.5	3.22	2.8	0.3g/kg/2 weeks
14	50	X-linked	villous atrophy	yes	n.a.	undetectable	2.24	4.7	FFP 400ml every 3 weeks
15	23	X-linked	hypoparathyroidism	yes	n.a.	undetectable	3.36	4.8	IV Sandoglobulin
16	19	X-linked	renal artery aneurysms	yes	n.a.	2.3	1.57	4.78	0.2g/kg/2 weeks
17	41	CVI	–	yes	n.a.	1.8	1.8	4.4	IV Gamimune
18	23	CVI	–	yes	n.a.	–	3.92	3.53	0.2g/kg/3 weeks
19	19	X-linked	bronchiectasis	no	n.a.	–	6.06	2.7	FFP 600ml every 2 weeks
20	41	CVI	epilepsy	yes	n.a.	2.6	2.86	4.2	IM IgG 750mg weekly
21		CVI	measles						
22		CVI	encephalitis						
23		CVI	malabsorption						
24		CVI	gall stones						
25		CVI	total parenteral nutrition						

CVI = Common variable immunodeficiency; X-linked = X-linked primary hypogammaglobulinaemia; NLH = nodular lymphoid hyperplasia; FFP = fresh frozen plasma; IM IgG = intramuscular immunoglobulin; IV Gamimune = intravenous immunoglobulin preparation (Cutter Labs); IV Sandoglobulin = intravenous immunoglobulin preparation (Sandoz); IDDM = insulin-dependent diabetes mellitus.

<sup>1</sup> All patients had a history of recurrent upper respiratory tract infections before the commencement of immunoglobulin replacement therapy.

<sup>2</sup> Clinical outcome of IV IgG therapy, defined as improvement in either respiratory or gastrointestinal symptoms compared with symptoms while on IM IgG therapy or on no IgG replacement therapy (n.a. = evaluation not applicable as patient did not have gastrointestinal symptoms).



bacterial infections with low serum IgG levels measured on at least two occasions at the time of diagnosis. All but 2 were already established on immunoglobulin replacement therapy before entry to the study. Further clinical details of individual patients are shown in table I.

Local hospital ethics committee consent, the consent of the patient's physician, and informed written consent from the patient were obtained prior to the study.

#### *SNBTS IV IgG*

The intravenous immunoglobulin used in this study (SNBTS IV IgG) is prepared at the Protein Fractionation Centre of the Scottish National Blood Transfusion Service (SNBTS) from a cold ethanol (Cohn) fraction II which is subsequently ultrafiltered, treated by mild pepsin proteolysis at pH 4 and freeze dried [8, 9]. The product after reconstitution with water is a solution of 5% (5 g/100 ml) human normal immunoglobulin.

#### *Method of Infusion*

**Infusion Dose and Rate.** A total SNBTS IV IgG dose of 200 mg/kg body weight was administered on most occasions. In 2 patients, cases 9 and 10, where the trough serum IgG level, i.e. immediately prior to IgG infusion, was not maintained above 3 g/l the infusion dose was steadily increased to 540 and 400 mg/kg, respectively.

In most patients the infusion was administered at a rate of 120 mg/kg/h. In patients who had never received any immunoglobulin replacement initially, this rate was reduced in the first infusions. The infusion rate was increased to 240 and 264 mg/kg/h for cases 10 and 9 without any adverse reactions.

#### *Frequency of Infusion*

Infusions were initially administered at 3-weekly intervals except in 3 patients (cases 9, 10, and 11) who received their infusions at 2-weekly intervals. Infusion frequency was modified according to the patient's clinical state while aiming to maintain a serum trough IgG level as near to the lower limit of normal as possible.

The infusion frequency was reduced from 3-weekly to 4-weekly (or even less frequently) if the trough serum IgG was above 4.0 g/l (normal range 5.0–13.0 g/l) and if the patient remained free from infection. If, however, the patient was clinically unwell and the trough serum IgG was below 3.0 g/l the frequency of infusion was increased to 2-weekly intervals. If the trough serum IgG was still below 3.0 g/l, the infusion dose was doubled or given at weekly intervals. Finally, if there was still no response a doubled infusion dose was administered at weekly intervals. The frequency of infusion therefore ranged from 1 week (cases 6, and 9) to 12 weeks (case 3).

A total of 517 infusions from 24 batches of SNBTS IV IgG were administered to the 17 patients over the study period.

#### *Methods of Assessment*

The oral temperature, pulse and blood pressure were monitored during the initial infusion at 0, 15, 30, 45, and 60 min and half-hourly thereafter until the end of the infusion. At later infusions when it was clear that patients tolerated their infusions, the recordings were less frequent.

Venous blood samples were collected from the infusion site at the commencement of the infusion and at the end of the infusion either from the opposite arm or from the infusion site after it had been flushed with 10 ml of blood. Serum IgG levels were assayed in the pre- and post-infusion samples.

IgG was measured by laser nephelometry [10]; the normal range for serum IgG concentrations (5.0–13.0 g/l) was determined by the measurement of IgG levels in 350 normal blood donors.

An attempt was made to evaluate the half-life of the infused IgG in SNBTS IV IgG in selected patients by collecting sequential samples from the patients after their first infusion. Assuming that the metabolism follows first-order kinetics, the half-life is proportional to the slope of the beta-phase and is determined by the formula:

$$\text{Half-life} = \ln 2/k = 0.693/k,$$

where  $k$  is the slope of the beta-phase determined by linear regression analysis [11, 12].

## **Results**

### *Serum IgG Concentrations following SNBTS IV IgG Therapy*

#### *Sequential Serum IgG Concentrations*

Figures 1–6 demonstrate sequential serum IgG levels before and after each infusion in selected patients. Pre-infusion samples give an indication of the trough level or lowest serum IgG level following SNBTS IV IgG infusion while post-infusion samples collected immediately following the immunoglobulin infusion are a reflection of the highest level that might be achieved following SNBTS IV IgG infusion.

In 13 of 17 patients who had received SNBTS IV IgG for more than 6 months, the serum trough IgG level was above 4.0 g/l within 8 months of the commencement of SNBTS IV IgG replacement therapy. In 4 of 5 patients who had received intramuscular IgG, the trough serum IgG level was higher than 4.0 g/l within 3 months of their initial infusion. Of the 2 patients who had never received any form of replacement therapy previously only 1 achieved a trough IgG concentration greater than 4 g/l within the first 3 months but both achieved that level within 8 months from the first infusion. Six of the 17 had a serum trough IgG level of greater than 5.0 g/l within 6 months of treatment.

Of the 10 patients who received treatment for at least 18 months, half of them maintained a trough serum IgG level of greater than 5.0 g/l for at least a year while 80% maintained their trough IgG above 4.0 g/l for at least 12 months. Seven maintained the trough IgG above 5.0 g/l for more than 3 months and 8 for more than 2 months.

Seven patients started treatment with SNBTS IV IgG from early 1983 and their sequential serum IgG levels

show the cumulative effect of intravenous immunoglobulin therapy as shown in case 11 (fig. 5). Two patients with bronchiectasis maintained their serum IgG level within the normal range. Some of the high serum IgG levels prior to commencing SNBTS IV IgG therapy were due to preceding therapy with fresh frozen plasma or another intravenous immunoglobulin preparation (e.g. case 3, fig. 1).

#### *Effect of Increasing Dose*

Two patients with X-linked primary hypogammaglobulinaemia (cases 9 and 10) who had persistently low serum IgG levels and who could not attend for infusions more often than once every 2 weeks had their infusion dose increased to 540 and 400 mg/kg. An increment in the serum IgG level was observed in only 1 patient (case 10, fig. 4). Attempts at further increasing the serum IgG level in case 9 consisted of increasing the infusion frequency (see below).

#### *Effect of Increasing Frequency of Infusions*

In 3 patients, IgG replacement therapy was carried out once every 2 weeks because of their low serum IgG despite previous replacement therapy with other intravenous immunoglobulin preparations (cases 9 and 11) and with fresh frozen plasma (case 10). In 1 further patient (case 6) the frequency of infusion was gradually increased to once weekly when he suffered from recurrent gastro-intestinal infections.

The serum IgG level in case 11 increased from 2.2 g/l to greater than 4.0 g/l after 3 months (fig. 5). The serum IgG level in case 10 who was receiving 800 ml of fresh frozen plasma every 3 weeks decreased from 3.2 g/l to around 2.0 g/l (fig. 5) while on intravenous IgG replacement regimen of 200 mg/kg every 2 weeks, presumably because of an actual decrease in total IgG infused via the latter replacement regimen. However, his serum IgG level gradually improved when his infusion dose was doubled. There was no significant increase in trough serum IgG level noted in case 9 (fig. 3). However, when his clinical state worsened attempts at further increasing his serum IgG levels were made by weekly infusions of IgG (see below).

In 1 patient (case 6), the serum IgG level reflected the severity of his gastro-intestinal disease (fig. 2). His serum IgG initially fell when he had diarrhoea. Increasing the frequency of SNBTS IV IgG from 3-weekly to 2-weekly and then to weekly intervals brought his trough serum IgG level into the normal range. However, with a worsening of his gastro-intestinal symptoms, his serum IgG level fell

again despite the increased frequency of the immunoglobulin administration. Following resection of the strictured and ulcerated segment of ileum found at laparotomy, his serum IgG levels were once again in the normal range while receiving replacement therapy at 3-weekly intervals. Histological examination of the resected bowel showed ulceration and lymphoid hyperplasia without any evidence of lymphoma or of Crohn's disease.

#### *Effect of Increasing Infusion Dose and Infusion Frequency*

One patient with X-linked primary hypogammaglobulinaemia (case 9) had his infusion dose as well as his infusion frequency increased when his clinical condition deteriorated. He had persistently low levels despite an increased dose of SNBTS IV IgG (300 mg/kg) and in frequency of treatment to fortnightly intervals. He has a combination of structural lung damage and gastro-intestinal disease as a result of recurrent infections. The infusion dose was increased to 540 mg/kg every 2 weeks without any significant increase in trough serum IgG concentration. However, at a later date, while an inpatient in hospital because of abdominal pain, he received 540 mg/kg at weekly intervals and his trough serum immunoglobulin level rose to 4.05 g/l (fig. 3).

#### *Effect of Reducing Infusion Frequency*

In 5 patients (cases 1, 3, 7, 12, and 17), the trough serum IgG level was maintained above 4.0 g/l when the frequency of infusion were increased to once every 4 weeks. With an infusion frequency of once every 4 weeks, patients (cases 1, 3, and 12) maintained their trough serum IgG level above 5.0 g/l. Figure 6 illustrates the effect of maintenance of serum IgG level with reduction in the frequency of infusion.

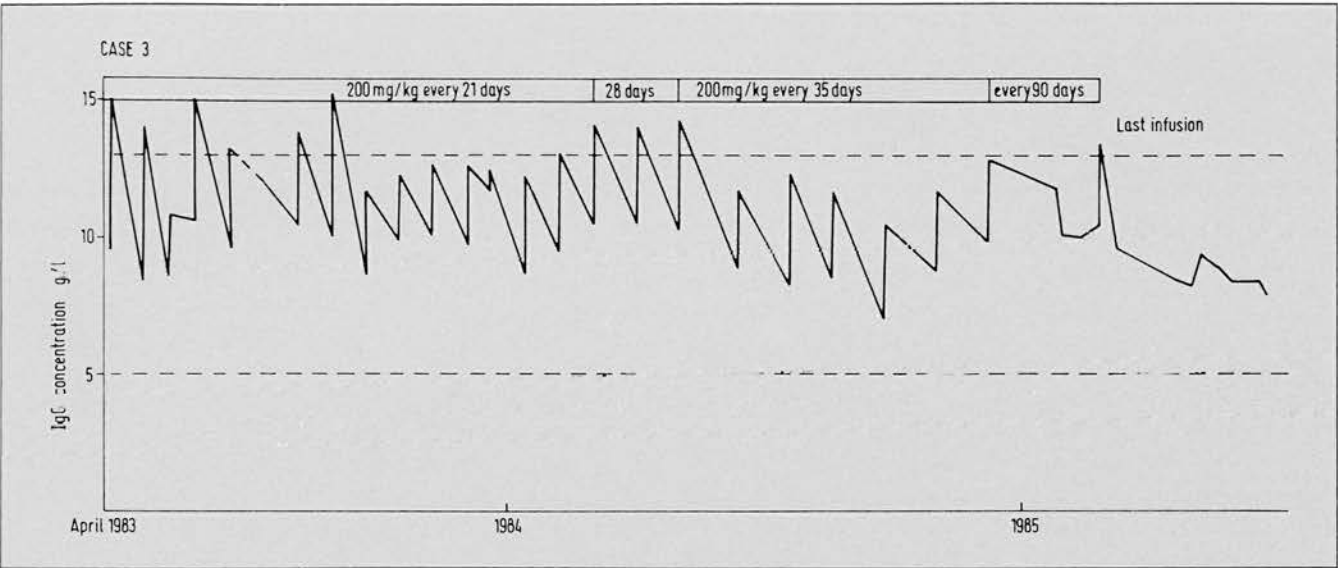
In 4 patients a trough serum IgG level of greater than 4.0 g/l was still achieved when the frequency of infusions was further reduced to once every 5 weeks (cases 1, 3, 7, and 12).

One patient (case 3) maintained a high trough serum IgG level of 8.49 g/l 16 weeks since her last infusion of SNBTS IV IgG of 200 mg/kg; the last but one infusion having been carried out 12 weeks previously (fig. 1).

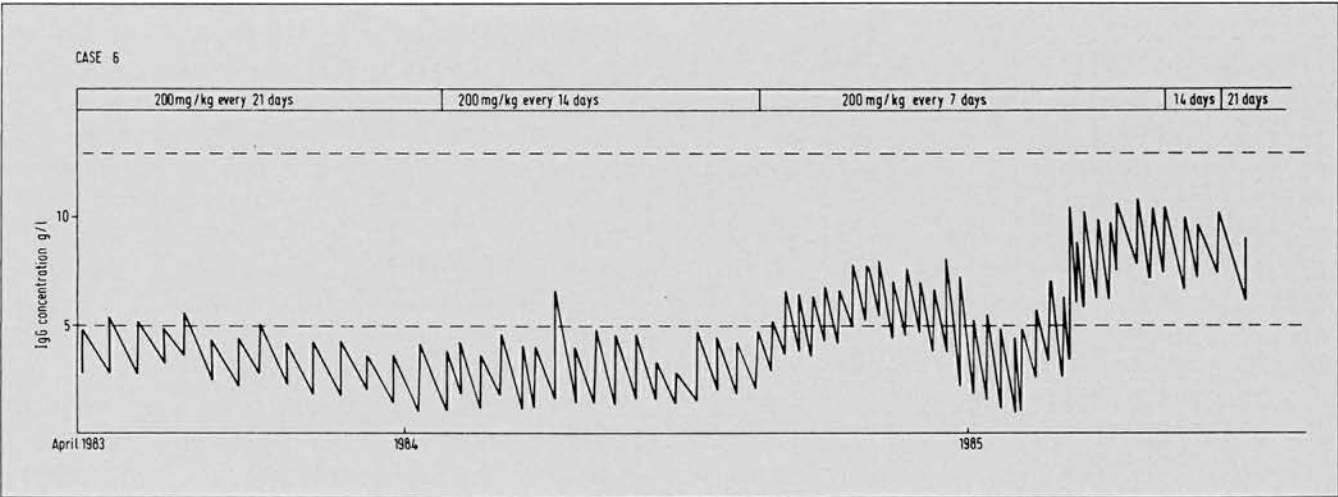
#### *Half-Life of Infused IgG*

Half-life values ranged from 15 to 53 days. No attempt was made to repeat the half-life measurements after a prolonged period of treatment.

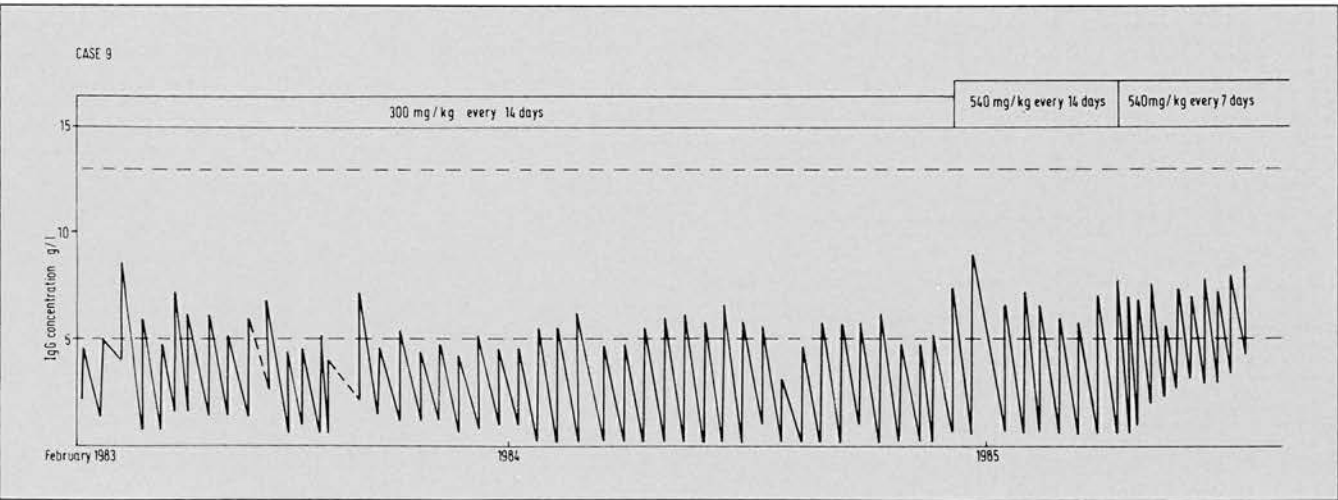
1



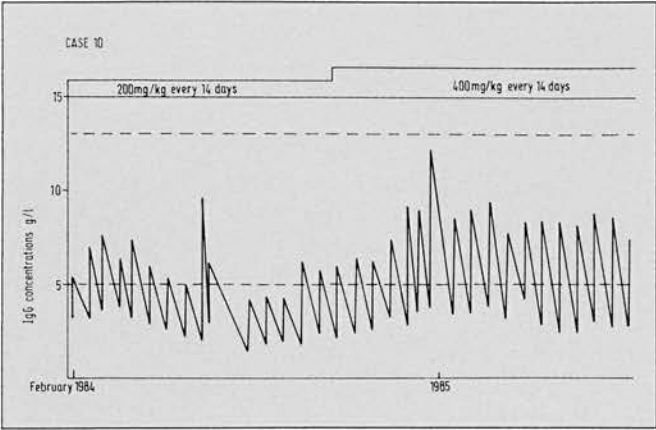
2



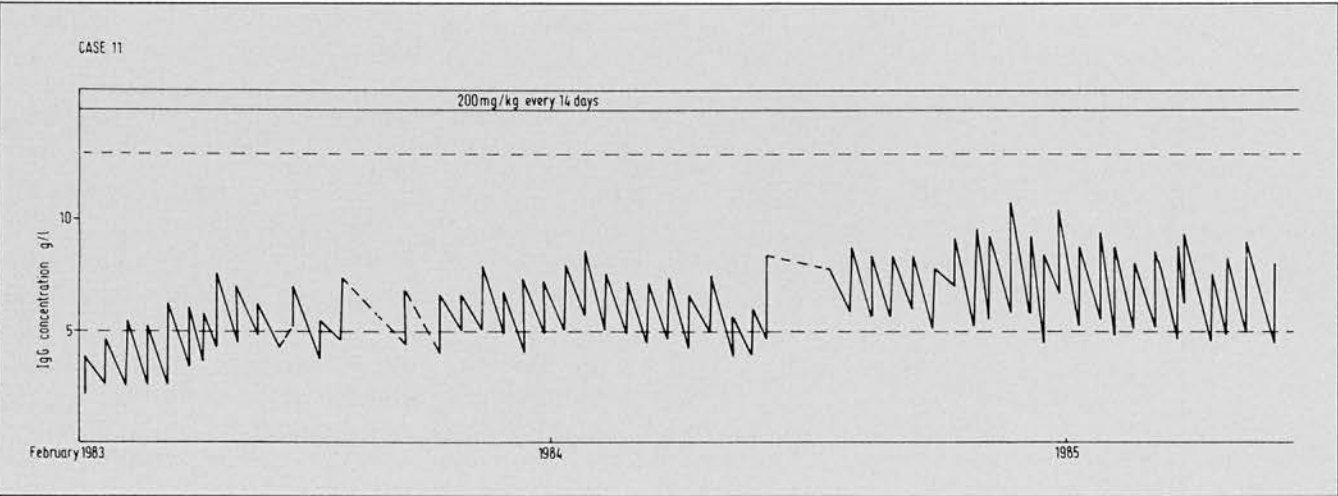
3



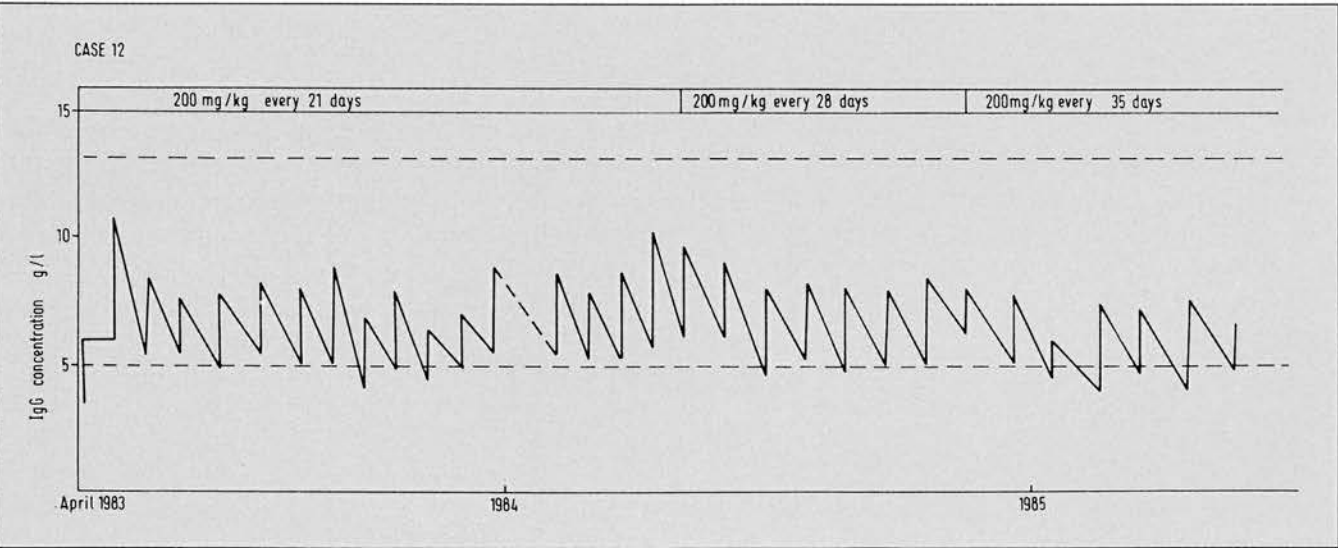
**Fig. 1-6.** The data of serum IgG measurement is shown on the horizontal axis and serum IgG concentrations on the vertical axis. The lower limit of the normal range for serum IgG concentrations (5 g/l) is shown by a horizontal broken line and the infusion dose and the frequency of SNBTS IV IgG infusion is shown at the top of each figure.



4



5



6



### *Adverse Reactions*

Adverse reactions were associated with 33 of 517 SNBTS IV IgG infusions (6.4%), and were mostly mild. Three adverse reactions were moderately severe, and occurred in the initial two infusions in 2 patients who had never previously received any form of immunoglobulin replacement.

Complement studies (total haemolytic complement CH50, C3, and C4) did not show any changes to suggest complement activation during any of the infusions including those where adverse reactions had been recorded.

### *Clinical Efficacy*

The clinical outcome of our patients in this study is documented in table I. Of the 13 patients with serum IgG levels above 4 g/l, all but 1 (case 5) experienced a reduction in the number of respiratory infections; case 5 had long-standing severe bilateral bronchiectasis. Case 16 with bronchiectasis continued to suffer from respiratory infections when his serum IgG level fell from 6.0 g/l while receiving treatment with fresh frozen plasma to 2.7 g/l following treatment with IV IgG.

The frequency of diarrhoea in 3 further patients (cases 2, 6, and 13) was unaffected when their serum IgG increased to above 4 g/l. Cases 9 and 10 with X-linked hypogammaglobulinaemia suffered from chronic diarrhoea despite serum IgG levels of 3.05 and 2.8 g/l, respectively. Finally, case 17 received total parenteral nutrition because of malabsorption, and therefore was free from diarrhoea.

### **Discussion**

Immunoglobulin replacement therapy for patients with primary hypergammaglobulinaemia has mainly been in the form of intramuscular injections. In the current recommended doses of immunoglobulin used (25–50 mg/kg/week) a proportion of such patients still suffer from recurrent infections and develop structural damage over a period of years [13]. We conducted this study to see if it was possible to maintain serum IgG concentrations at a normal or near normal level over a long period of time using an intravenous immunoglobulin preparation.

Patients with primary hypogammaglobulinaemia usually have serum IgG of 2 g/l or less before treatment.

With intramuscular immunoglobulin therapy at 25 mg/kg/week, the response is variable but the serum IgG levels are only raised by 1–2 g/l with the occasional patient showing no increment in IgG level at all. The serum IgG levels achieved in individual patients are variable and argue strongly against the practice of administering the same dose of IgG to all immunodeficient patients [14]. The half-life of infused IgG is very variable among such patients, and clinical observations have suggested that it is shorter during intercurrent infection [15]. There is therefore a need to individualise the dosage of infused IgG in patients with hypogammaglobulinaemia.

Attempts to individualise treatment dosage should achieve a high enough trough serum IgG level to minimise the incidence of infection, using the minimal quantity of IgG required due to cost and with a minimum number of infusions to enable the patient to lead as normal a life as possible. However, there is conflicting data on the optimal serum IgG level that such patients need to maintain in order to minimise the risk of infection. In one study by Janeway and Rosen [16], a serum IgG level of about 2 g/l markedly reduced the incidence of serious infections. In contrast, Joller et al. [17] have concluded that a minimal serum IgG concentration of about 3 g/l in patients with hypogammaglobulinaemia is associated with an acceptably low risk of acute infections. As no definitive clinical trials have been set up to determine the optimum IgG level for protection against infection and other complications of hypogammaglobulinaemia most clinicians aim to achieve a near normal serum IgG level in their patients since it is likely that the nearer to normal the serum IgG level, the lower the incidence of infection.

Previous studies have attempted to individualise the dose of immunoglobulin to achieve a satisfactory serum IgG concentration but only one study increased the frequency of IgG infusions. Morell et al. [18] demonstrated that the administration of 300 mg/kg/month to patients with antibody deficiency could increase and maintain their serum IgG level between 3.0 and 5.0 g/l. Higher serum levels can be achieved by disproportionately larger doses of IgG administered at short intervals, and sustained therapeutic IgG levels can be obtained with 500 mg/kg of IV IgG [19]. However, while most studies have shown satisfactory increments in serum IgG levels following administration of 300 mg/kg/month, there are still patients who have persistently low serum IgG levels [5]. In one study, Schiff et al. [7] used the half-life of infused IgG to determine the dose required to maintain trough IgG levels above 2.0 g/l in his patients with humoral immunodeficiency. There was no reduction in the

number of infective episodes when the patients received the higher dose of IgG, but this was probably because the trough serum IgG level achieved was only around 2.0 g/l [7].

We have found that the serum IgG levels achieved with SNBTS IV IgG therapy at a dose of 200 mg/kg 2 or 3 times weekly were excellent with trough levels in the normal range (greater than 5 g/l) for over a year in 50% of our patients. Moreover, by increasing the infusion dose or by reducing the interval between two infusions or by a combination of the two we have managed to achieve and maintain substantial increments in the trough IgG level in our patients. Sequential serum IgG levels in case 6 demonstrate the increased losses of infused IgG in the gastrointestinal tract. In such patients adequate levels can be maintained in the near normal range with frequent infusions of IgG with the usual dose of 200 mg/kg. Similar increases could also be achieved in patients by using very high doses; the equivalent of 8 times the usual dose of IV IgG as in case 9. When the intervals between infusions were increased from 3 to 5 weeks in 6 selected patients the trough serum IgG level was maintained at above 4.0 g/l. One patient (case 3) maintained her serum IgG level in the normal range for 16 weeks after her last infusion. Although she had primary panhypogammaglobulinaemia with a history of recurrent bacterial infections associated with very low IgG levels, it is possible that she is now producing antibodies. Further investigations are in progress.

In summary, 76% of our patients achieved a trough serum IgG level of at least 4.0 g/l within 8 months of the start of SNBTS IV IgG and 80% of patients maintained their trough serum IgG level above 4.0 g/l when they received SNBTS IV IgG in a dose of 200 mg/kg every 4–5 weeks. In patients with large immunoglobulin losses, normal trough serum IgG levels can be safely achieved with the use of larger doses of SNBTS IV IgG by increasing the infusion doses or the frequency of infusions or by increasing both the frequency and dose of infusions. While most of our patients including those with mild to moderate bronchiectasis improved during the study, we have been unable to influence the frequency of diarrhoea in our patients, and 1 patient with long-standing severe bilateral bronchiectasis continued to suffer from recurrent pyogenic respiratory infections.

### Acknowledgements

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## **Double-blind comparative trial of standard (commercial) and antibody-affinity-purified tetanus toxoid vaccines**

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### **Summary**

Tetanus toxoid purified by antibody-affinity chromatography, was compared with conventionally purified material in a double-blind trial in 205 healthy blood donors. There was neither any difference in immunogenicity as assessed by enzyme-linked immunoassay nor in side-reactions between the two vaccines. This study confirms that side-reactions to tetanus toxoid are not eliminated by purifying it.

### **Introduction**

Local and mild systemic side-reactions after administration of tetanus toxoid are quite common.<sup>1</sup> Furthermore, the frequency and severity of such reactions increases with the number of doses given.<sup>2</sup> These reactions may be due to hypersensitivity to the toxoid or to proteins of *Clostridium tetani* which co-purify with toxoid during the precipitation process used in its conventional preparation. It was, therefore, thought possible that a purer toxoid might be less reactive.

A purified tetanus toxoid containing fewer contaminant proteins has been prepared by antibody-affinity chromatography. This study was designed to compare the purified with conventional toxoid in terms of the antitoxin response and clinical reactivity in adult vaccinees.

### **Methods**

#### **Vaccinees**

A total of 205 healthy adult volunteers of both sexes aged 18-64 years were recruited from blood donors attending the Scottish National Blood Transfusion Service, Edinburgh. Exclusions from the study were: (i) administration of Tetanus Immune Globulin within 6 weeks before the study; (ii) administration of tetanus toxoid within 12-months before the study; (iii) pregnancy; and (iv) subjects who had experienced a severe reaction to a previous dose of tetanus vaccine. Vaccination and reaction histories were determined by interview. Initial venous blood samples were provided for assay of tetanus antitoxin. Volunteers, matched for age and sex (Table I) and, with six

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Table 1 *Characteristics of recipients of conventionally purified (CPT) and antibody-affinity-purified (AAPT) tetanus toxoid vaccines*

	CPT	AAPT
No. in group (% males)	102 (50 %)	103 (51 %)
Mean age (range) in years	34 (18-64)	32 (18-64)

exceptions, tetanus antitoxin titres, were randomly assigned to receive conventionally purified toxoid (CPT) ( $n = 102$ ) or antibody-affinity purified ( $n = 103$ ) toxoid (AAPT). A single 0.5 ml dose of vaccine was administered by means of a syringe and needle to each volunteer by deep subcutaneous injection. All doses were selected by reference to a list assigning volunteers' names to treatment numbers. Doses were, therefore, administered on a double-blind basis.

All volunteers gave informed consent to participation in the study. The protocol was approved by the Ethical Committee of the Scottish National Blood Transfusion Service.

### Vaccines

CPT (Absorbed tetanus vaccine, Wellcome lot IPPT 31) contained 10  $L_f$  of tetanus toxoid (1770  $L_f$ /mg protein N) and had an immunising potency of 80.2 IU/dose. AAPT (Wellcome lot IPPT 27) contained 10  $L_f$  of tetanus toxoid (2317  $L_f$ /mg protein N) and had an equivalent immunising potency (109.4 IU/dose). AAPT was purified by antibody-affinity chromatography and the use of equine tetanus antitoxin conjugated to Sepharose by the cyanogen bromide method. Both vaccines contained 0.69 mg  $Al^{3+}$ /dose.

### Clinical reactions

Vaccinees were asked to complete a daily record for 7 days after vaccination so as to record any reactions at the site of injection (pain, redness, tenderness and the presence of a nodule) as well as systemic reactions (headache, feverishness and malaise). Pain, redness, and tenderness were graded 1-3 according to whether they were considered mild, moderate or severe. Volunteers recorded their morning oral temperature daily for 7 days after immunisation.

### Measurement of tetanus antitoxin

Blood was collected from 204 volunteers before and approximately 2 weeks after immunisation. One recipient of AAPT failed to provide paired serum samples. The concentration of circulating tetanus antitoxin was determined on paired pre- and post-immunisation serum samples by ELISA for specific IgG antibodies (G. R. Barclay, unpublished). The concentration of tetanus antitoxin in a subset of vaccinees was also measured by means of the toxin neutralising test of Glenny and Stevens.<sup>3</sup>



Table II *Local reactions reported by participants in a clinical comparison of conventionally purified (CPT) and antibody-affinity-purified (AAPT) tetanus toxoid*

Vaccine	CPT	AAPT
No. of participants	102	102
Any local reactions		
No. (%) reporting	76 (75)	83 (81)
Mean no. of days* (range)	4.14 (1-7)	4.17 (1-7)
Redness		
No. (%) reporting	54 (53)	53 (52)
No. (%) severe reactions	5 (5)	5 (5)
Mean no. of days* (range)	3.30 (1-7)	3.57 (1-7)
Pain		
No. (%) reporting	30 (29)	29 (28)
No. (%) severe reactions	0 (0)	1 (1)
Mean no. of days* (range)	2.47 (1-5)	2.28 (1-7)
Tenderness		
No. (%) reporting	62 (61)	61 (60)
No. (%) severe reactions	5 (5)	1 (1)
Mean no. of days* (range)	2.95 (1-7)	2.85 (1-6)
Lump at site of injection		
No. (%) reporting	49 (48)	53 (52)
Mean no. of days* (range)	4.41 (1-7)	4.30 (1-7)
Other reports†		
No. (%) reporting	23 (23)	18 (18)

\* Total number of person days on which reactions were reported.

† Itch; bruise; swelling.

## Statistics

Statistical evaluation of significance of differences in frequencies of events between groups was determined by Fisher's exact test.

## Results

### Clinical reactions

Calendar records were returned by 204 participants in the study. One recipient of AAPT failed to return a reaction card.

Inspection of the calendar records did not reveal any significant reaction to either vaccine. Only transient mild systemic symptoms and signs with mild discomfort at the site of injection were reported. No significant difference in the overall frequency of reactions was detected between the groups with 83/102 (81%) of CPT recipients as compared with 88/102 (86%) of AAPT recipients recording any reactions during the study ( $P > 0.2$ ).

The frequency of local reactions, defined as redness, pain, tenderness, and the presence of a lump at the injection site, are shown in Table II. The frequency of local reactions reported in the recipients of CPT was 76/102 (75%) as compared with 83/102 (81%) in the group receiving AAPT. The

Table III Systemic reactions reported by participants in a clinical comparison of conventionally purified (CPT) and antibody-affinity-purified (AAPT) tetanus toxoid

Vaccine	CPT	AAPT
Any systemic reactions		
No. (%) reporting	29 (28)	29 (28)
Mean no. of days* (range)	2.28 (1-7)	1.93 (1-7)
Pyrexia ( $> 37.5^{\circ}\text{C}$ )		
No. (%) reporting	5 (5)	5 (5)
Mean no. of days* (range)	1.80 (1-4)	1.50 (1-2)
Headache		
No. (%) reporting	14 (14)	14 (14)
Mean no. of days* (range)	2.36 (1-6)	1.64 (1-7)
Feverishness		
No. (%) reporting	4 (4)	6 (6)
Mean no. of days* (range)	2.00 (1-3)	1.83 (1-3)
Miscellaneous reports†		
No. (%) reporting	13 (13)	12 (12)
Mean no. of days* (range)	2.1 (1-6)	1.4 (1-3)
Other reactions‡		
No. (%) reporting	5 (5)	7 (7)
Mean no. of days* (range)	2.0 (1-3)	2.1 (1-7)

\* Total number of person-days on which reactions were reported.

† Possible vaccine-associated reactions: nausea, dizziness, malaise, peripheral paraesthesia.

‡ Diarrhoea, abdominal pain, urinary frequency, upper respiratory tract infections.

difference is not statistically significant. Further details of the incidence of local reactions are shown in Table II.

In the 7 days following vaccination, 29 (28%) CPT recipients and 29 (28%) AAPT recipients experienced systemic symptoms or signs that might have been associated with the vaccines (Table III). These included fever (temperature  $\geq 37.5^{\circ}\text{C}$ ), headache, influenza-like symptoms, nausea, dizziness, malaise, myalgia, or peripheral paraesthesia. Other symptoms such as diarrhoea, abdominal pain, urinary frequency and sore throat may have been related to concurrent infections. They were therefore categorised separately. One recipient of CPT reported peripheral paraesthesia on the 2nd and 3rd days after vaccination. It is unclear whether this arose in the injected or contralateral arm. Analysis of the frequency of symptoms and signs did not demonstrate any statistically significant difference between the two vaccine groups.

### Antitoxin response

The increases in tetanus antitoxin as assessed by ELISA, in the recipients of each vaccine are shown in Tables IV and V. Of 59 recipients of CPT 44 (75%) and of 59 recipients of AAPT 45 (76%) with previously undetectable antitoxin developed concentrations indicative of immunity to tetanus, i.e., at least 0.01 IU/ml. Altogether, 83 CPT recipients (81%) and 86 AAPT recipients (84%) had an increase in tetanus antitoxin following immunisation. There was no difference in either the frequency or magnitude of antibody response in the two groups.

Table IV *Serological responses to antibody-affinity-purified (AAPT) and conventionally purified (CPT) tetanus toxoid as assessed by ELISA*

	CPT	AAPT
Total no. assessed	102	102
Pre-immunisation tetanus antitoxin value = 0	59	59
Post-immunisation tetanus antitoxin value = 0	15	14
No. with an increase in tetanus antitoxin	83	86
GMT* pre-vaccination	1.48	1.52
post-vaccination	6.21	6.65

GMT = Geometric mean tetanus antitoxin titre calculated on the assumption that antitoxin titres were below the limits of detection, i.e., 0 taken to equal the lowest possible titre.

Table V *Increase in tetanus antitoxin*

	CPT	AAPT
> 2.5 IU/ml	12	15
2.5-5.0 IU/ml	7	15
5.0-10.0 IU/ml	29	23
10.0-20.0 IU/ml	23	20
20.0-40.0 IU/ml	12	11
40.0-80.0 IU/ml	0	2
Total	83	86

Analysis of magnitude of increase in tetanus antitoxin titre measured by ELISA

CPT, conventionally purified tetanus toxoid vaccine; AAPT, antibody-affinity-purified tetanus toxoid vaccine.

### Discussion

There was no difference in side effects and immunogenicity between the two vaccines. In this study the overall incidence of local reactions (83.8%) was higher than that found in a smaller study by Deacon and colleagues (68%) in which a similar method of recording reactions was used.<sup>1</sup> Of the 29 non-immune vaccinees who failed to develop antibodies to the toxoid, 18 had no memory of any tetanus vaccination, seven said that they might have been vaccinated during schooldays but not in the last 25 years, and four clearly remembered initial vaccination or 'booster' doses but not within the last 10 years; 24 of the 29 were at least 30 years of age. It may be that many of these non-responders may not have been able to mount a secondary immune response since they would not necessarily have received routine primary tetanus immunisation in infancy.

The lack of any significant difference between the effects of the two vaccines in this study together with the lower incidence of reactions with plain formol tetanus toxoid<sup>4, 5</sup> suggest that local reactions may be due to the toxoid itself



and/or to inflammatory responses to aluminium hydroxide rather than to the major impurities that are co-purified with the toxoid in the conventional purification technique and which are removed by antibody-affinity purification. This is consistent with the results of previous studies.<sup>2, 6</sup>

Development and evaluation of a pure toxoid devoid of contaminating proteins cross-linked by the toxoiding process will continue. It is possible, however, that the current local and mild systemic reactions are an inescapable consequence of repeated administration of tetanus toxoid. Nevertheless, different analyses of the data yielded by this study suggest that there is no greater frequency or severity of reactivity associated with high values of preimmunisation tetanus antitoxin. Conversely, higher reactivity may arise in association with low concentrations of preimmunisation antibody.<sup>7</sup> The status of the vaccinees' immunity to tetanus which may determine whether a reaction arises after administration of tetanus toxoid is evidently complex and requires further investigation. Even so, evidence suggests that the risk of a reaction does not appear to increase with increasing amounts of antibody.<sup>7</sup> This does not support the widely held view that repeated 'booster' doses lead to an increased risk of reactions.

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